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IMMUNOFLUORESCENCE, AN ANNOTATED BIBLIOGRAPHY.
II. VIRAL STUDIES

Warren R. Sanborn

Fort Detrick
Frederick, Maryland

March 1968

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MISCELLANEOUS PUBLICATION 20

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MISCELLANEOUS PUBLICATION 20

IMMUNOFLUORESCENCE, AN ANNOTATED BIBLIOGRAPHY

II. VIRAL STUDIES

Warren R. Sanborn

March 1968

Technical Information Division
AEROBIOLOGY AND EVALUATION LABORATORY

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FOREWORD

The use of immunofluorescence, or fluorescent antibody, was initiated by Dr. Albert H. Coons and his co-workers in 1942. Dr. Coons has modestly stated that making antibodies fluorescent was "simply another variation of their use as reagents for the identification of specific antigen. . . ." However, this "variation" has proved to be one of immense significance to modern immunology. Its importance lies in the wedding of the two broad areas of investigation, morphology and immunology, thus allowing the detection of immunologic reactions at the cellular level.

The volume of literature related to immunofluorescence or fluorescent antibody and covering use of this technique has expanded explosively over the relatively few years since its inception. This expanding literature volume bears witness to the basic value of the technique. Through 1954, only about 40 articles had been published. In the next two years, 58 were added. During 1957 and 1958 there were 83 and 96, respectively. By 1961 the annual figure had reached more than 260 articles. For this supplementary second edition, the figures for 1963, 1964, and 1965 are 551, 764, and 678, respectively. These totals are testimony to Dr. Coons' genius.

Although it would be virtually impossible to cite every article that refers to the use of immunofluorescence, an attempt has been made to approach that limit. To that end, more than 445 journals were searched. In addition, six abstracting journals and the computer system of the National Library of Medicine, MEDLARS, were employed. Fifteen languages are represented. Translations were provided by colleagues of the compiler, government translating services, abstractors, and the compiler. The earliest entry in the original edition was 1905. In the present edition, entries covering the years 1963, 1964, and 1965 are the primary ones included, but there are also a few earlier entries not listed in the first edition. Further entries for 1966 and 1967 are now being compiled; these will be incorporated into further revisions of this bibliography.

The bibliography is intended to aid investigators in following the expanding mass of literature on the technique and to improve their skill in its use. This entire second edition, Miscellaneous Publication 20, has the same overall title, "Immunofluorescence, an Annotated Bibliography," as the first edition (Miscellaneous Publication 3). The present edition also has the same six-volume structure: Volume I, "Bacterial Studies"; Volume II, "Viral Studies"; Volume III, "Studies of Fungi, Metazoa, Protozoa, and Rickettsiae"; Volume IV, "Studies of Animal Physiology"; Volume V, "Diagnostic Applications and Review Articles"; and Volume VI, "Technical Procedures." Each of the volumes is subdivided into subject categories that should, hopefully, aid the reader in finding pertinent information in his field of interest without his spending undue time in scanning superfluous citations. Articles within subject categories are arranged alphabetically by senior author. A seventh volume, "Author and Subject Indexes," has been added to further aid the investigator in his search for articles relevant to his interest area.

Abstracts for citations in this edition have been prepared or modified in keeping with the central theme, the application of immunofluorescence to various problems. If the primary emphasis in the original article was immunofluorescence and the author's summary reflected this, the summary was generally left unchanged, except for minor changes and abbreviations simply to save space. In other instances, it was necessary to write a new abstract in order to indicate the proper place of immunofluorescent technique in the study. At the same time, the main point of such articles was maintained in abbreviated form in the abstract. Hopefully, this approach will be successful in bringing the application of immunofluorescence to the attention of the reader, while preserving each author's ideas at the same time. It is further hoped that this bibliography will aid investigators in avoiding duplication of effort and thus contribute to even greater and more imaginative applications of immunofluorescence.

Accession numbers have been assigned consecutively to citations throughout all six volumes of this edition. The plan for further future volumes allows this simple system. Entries applicable to more than one subject category appear more than once, and these will have an accession number for each placement in each volume.

A complete author index is included in each volume; the author's name is listed with the accession numbers of the entries with which he is associated. The asterisk designates those for which he is senior author.

To avoid excess duplication and unwieldy size, the second parts of Volumes V and VI contain only basic citations for articles printed in the other four volumes. However, titles of articles are included to assist the reader in selection of those citations of possible interest. As in the other volumes, the references are placed in subject categories and are arranged alphabetically by senior author within categories. The authors, the year of publication, and the volume and accession number are shown to indicate where the complete entry can be found.

For brevity, certain abbreviations in common usage in this field have been used rather than the more ponderous forms. For unmistakable identification, they are listed below.

| | |
|---------|-----------------------------------------------------|
| BSA | bovine serum albumin |
| DANS | a. 1-dimethylaminonaphthalene-5-sulfonic acid |
| | b. 5-dimethylamino-1-naphthalene sulfonic acid |
| | or chloride form. |
| FA | fluorescent antibody |
| FIC | fluorescein isocyanate |
| FITC | fluorescein isothiocyanate |
| FTA | fluorescent treponemal antibody |
| FTA abs | fluorescent treponemal antibody absorbed |
| FTA-200 | a modification of the above based on serum dilution |
| PAP | primary atypical pneumonia |

| | |
|--------|------------------------------------------------------------------------------------------------------------------------------------------|
| PAS | para-aminosalicylic acid |
| PBS | phosphate-buffered saline |
| RB 200 | a. lissamine rhodamine RB 200 b. lissamine rhodamine B 200 c. lissamine rhodamine B d. sulphorhodamine B e. acid rhodamine B |
| TPFA | <u>Treponema pallidum</u> fluorescent antibody |
| TPI | <u>Treponema pallidum</u> immobilization |

Generally, the citations follow the format prescribed by the second edition of Style Manual for Biological Journals, American Institute of Biological Sciences, 2000 P Street, N.W., Washington, D.C., 20036. Abbreviations follow "American Standard for Periodical Title Abbreviations," Z39.5-1963, American Standards Association Incorporated, New York.

The compiler began to collect this immunofluorescence literature in 1957 while he was stationed at U.S. Navy Preventive Medicine Unit No. 2, Norfolk, Virginia. The literature collection became more intense and organized after 1959 when he was transferred to Fort Detrick, Frederick, Maryland. Following his further transfer to the Microbiology Department of the Naval Medical Research Institute, Bethesda, Maryland, in 1963, he continued this work with the encouragement and support of both of these latter installations. Work on the second edition began in 1964, and it has continued through support from both the U.S. Army and the Bureau of Medicine and Surgery of the U.S. Navy. This volume was completed while the compiler was assigned to U.S. Navy Medical Research Unit No. 3, FPO, New York, 09527, where he is currently serving as head of the Bacteriology Department.

The information in these volumes was originally recorded on coded marginal punch cards. With the compilation of this publication, the citations and annotations have been transcribed on punched tape for conversion to automatic data processing and for use in updating later editions. Each entry is coded for recall by authors, date, title, and source publication to allow compilation of more selective listings.

Readers are invited to report errors or suggest added entries to the compiler or to Editorial Branch, Technical Information Division, Fort Detrick, Frederick, Maryland, 21701, for improvement of the subsequent editions. Reader assistance in this area will be deeply appreciated.

ACKNOWLEDGMENTS

The essential team effort required for development of this immunofluorescence bibliography cannot be overstressed. As with many projects of this nature, the talents, advice, guidance, and assistance of many people led to the completion of this second edition. The compiler is deeply grateful to the many people who have contributed.

Financial support for this project at first was absorbed by the Pathology Division and the Walter Reed Army Medical Unit, Fort Detrick. However, completion of the first edition (through 1962) was made possible by special financial assistance from Physical Defense Division, Fort Detrick, under Dr. Charles R. Phillips. I am extremely grateful to him for his aid. Expenses for this second edition were primarily met through a generous grant from U.S. Navy Bureau of Medicine and Surgery, Preventive Medicine Division, under CAPT J. Millar, MC, USN. Many administration expenses also were borne by the Naval Medical Research Institute and by Fort Detrick.

A number of libraries kindly donated their services. In spite of the unusual requests required by this project, these libraries were very helpful and willingly assisted, often providing valuable suggestions. Libraries primarily involved were the Technical Library, Fort Detrick,, under Mr. Charles N. Bebee and later Miss Joyce A. Wolfe, and the Technical Reference Library, Naval Medical Research Institute, Mrs. T.P. Robinson, librarian. Much valuable assistance also was rendered by the National Institutes of Health Library, Miss R. Connelly, reference librarian, the National Library of Medicine, and the library of the Walter Reed Army Medical Unit, Fort Detrick. The staff members of these libraries were both helpful and patient. Without such fine assistance, the work could not have been completed.

It is a pleasure to acknowledge the highly competent secretarial help. Secretaries providing their capable and untiring talents were: Miss Sandra Rosenblatt, Miss Linda L. Zimmerman, Mrs. Marguerite M. Matovich, Mrs. Gene Heaven, Mrs. Linda Franklin, Mrs. Alberta Brown, Mrs. Margaret Raheb, and a number of others. Valuable assistance in double-checking problem references was provided by Mrs. Catherine F. Eaves and Mrs. Mary J. Gretzinger. Dr. George H. Nelson was a willing consultant for classification problems. Dr. Harold W. Batchelor provided an essential key to the development of this work by introducing the compiler to marginal punch card systems and guiding him in their application.

The Technical Information Division, under Mr. Gerald W. Beveridge, continually provided all types of assistance in addition to a home base from which to work. My gratitude for this cannot be fully expressed.

Last, but by no means least, the essential editorial work receives my highest praise. The tireless efforts, patience, and driving force supplied by these people were the prime factors in bringing this edition to completion. Mrs. Madeline Warnock Harp, in charge, Mrs. Mary D. Nelson, and Mrs. Ruth P. Zmudzinski all spent many hard weeks of work on this project. I shall always be indebted to them.

ABSTRACT

This volume is one of a series of six in the second edition of an annotated bibliography on various aspects of immunofluorescence and its use. The first six-volume edition was published in 1965 and included citations for the period 1905 through 1962. The present edition covers the period 1963 through 1965; Volume II contains 441 annotated literature citations, arranged according to major subject areas, and a complete author index.

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I. ARBOVIRUS

5491

Albrecht, P. 1965. Detection of group B arboviruses in chick embryo cell cultures by the fluorescent antibody method. *Acta Virol.* 9:338-346.

Six group B arboviruses, namely tick-borne encephalitis, Japanese B encephalitis, West Nile, St. Louis encephalitis, dengue, and yellow fever viruses, were tested for their multiplication capacity and sensitivity of detection in chick embryo cell (CEC) cultures. Only tick-borne encephalitis virus could be readily passaged in CEC cultures, whose sensitivity to its detection by immunofluorescence or by reinoculation to mice was about equal to that of parallel titration in mice. Japanese B encephalitis, West Nile, and St. Louis encephalitis viruses, though not passageable in CEC cultures, were detected with higher sensitivity in the first CEC culture passage by immunofluorescence or by reinoculation to mice than in intracerebrally inoculated mice. CEC cultures proved unsuitable for demonstration of dengue and yellow fever viruses by either of the above-mentioned techniques. The morphology of, and fluorescent antigen distribution in, CEC cultures infected with the six group B arboviruses are described.

5492

Avakian, A.A.; Altshtein, A.D. 1964. A study of acute and chronic infection due to tick-borne encephalitis virus in tissue culture: III. A possible mechanism of chronic infection. *Vop. Virusol.* 9:575-580. In Russian.

In a system with an acute form of infection, tick-borne encephalitis virus in swine embryo kidney cells, the proportion of cells maintaining the virus multiplication and revealed by using infective centers and immunofluorescence methods is higher than in systems with a chronic form of infection. Virus-maintaining cells are destroyed irrespective of the form of infection at the level of the cell population. The cytopathic effect in systems with a chronic form of infection is not detectable because of a small proportion of such cells, different times of their degeneration, and their rapid removal from the monolayer in the process of destruction. A hypothesis of the mechanism of acute and chronic infection caused by tick-borne encephalitis virus in tissue culture is discussed.

5493

Bhamarapravati, N.; Halstead, S.B.; Sookavachana, P.; Boonyapaknavik, V. 1964. Studies on dengue virus infection: I. Immunofluorescent localization of virus in mouse tissue. Arch. Pathol. 77:538-543.

The application of fluorescent antibody to tissue sections was used in the study of dengue infection in newborn mice. Specific fluorescence appears about 72 to 96 hours after inoculation. The antigen was localized mainly in the cytoplasm of neurones and glial cells in the brain. The first appearance of antigen precedes evidence of neuronal damage and clinical disease. Cross-staining of antibody to dengue 1 antigen with all types of dengue antigen as well as with Japanese encephalitis virus antigen was observed. The indirect method of staining can be used to study antibody conversion in patients who have Thai hemorrhagic fever. Dengue antigen can be demonstrated in acetone- or formalin-fixed, paraffin-embedded sections.

5494

Blaskovic, D. 1964. Arbovirus encephalitides in Europe. Rendic. Ist. Super. Sanit. 26:121-136.

FA demonstrated that tick-borne encephalitis virus multiplies in cytoplasm of nerve cells throughout the CNS. Vivid fluorescence was seen in the olfactory mucosa and retina ganglia cells. Other cells containing virus are described. Virus was also found in various endodermal and mesenchymal cells. Estimations are made on the mode of spread in the host.

5495

Easterday, B.C.; Jaeger, R.F. 1963. The detection of Rift Valley fever virus by a tissue culture fluorescein-labeled antibody method. J. Infect. Dis. 112:1-6.

The fluorescent antibody technique was used to detect Rift Valley fever virus (RVF) antigen in hamster kidney and Chang human liver cell cultures. The technique was also used to detect RVF antigen in tissues from infected lambs and mice. Monkey anti-RVF immune globulin conjugates were superior to rooster and lamb immune globulin conjugates for staining infected tissues. Infected culture cells and animal tissue cells were unequivocally differentiated from noninfected materials. The fluorescent staining was always in the cytoplasm, with no evidence of nuclear staining. RVF antigen was detected in the cytoplasm of hamster kidney cells after 5 hours of incubation at 37 C when a virus-cell ratio of about 5:1 was used. A tissue culture system combined with the fluorescent antibody technique could provide a rapid means of diagnosis for RVF.

5496

Hannoun, C.; Fernandes, M.V.; Macieira-Coelho, A. 1965. Effects of cortisone on in vitro viral infection: I. Inhibition of cytopathogenesis by polio, rabies, and yellow fever viruses in human embryonic cells. *Proc. Soc. Exp. Biol. Med.* 119:153-158.

Plaque formation, observation of cytopathic effect, immunofluorescence, and mouse inoculation were used variously to evaluate some aspects of cortisone acetate on infection of diploid human embryonic fibroblastic cultures with polio, rabies, and yellow fever viruses. Cortisone did not affect poliovirus directly, but did inhibit plaque production when cells were subjected to many passages in its presence. Both accumulation of cytopathic effect and spread of virus in cultures infected with rabies virus before serial subculture were inhibited by cortisone in passage medium. Cortisone exerted a similar cumulative effect on virus production by cells infected with yellow fever virus, and an immediate inhibition of cytopathic response, but both virus production and cytopathic effect returned on removal of cortisone from the medium. The findings suggested a cumulative but nonselective effect of cortisone on cellular capacity to respond to virus infection.

5497

Henle, W. 1963. Interference and interferon in persistent viral infections of cell cultures. *J. Immunol.* 91:145-150.

This paper discusses virus-cell interaction that permits maintenance of chronically virus-infected continuous cell lines. Numbers of infected cells have been assessed by immunofluorescence as well as by other methods. Immunofluorescence is particularly well suited to such an application.

5498

Johnson, R.T. 1965. Virus invasion of the central nervous system: A study of Sindbis virus infection in the mouse using fluorescent antibody. *Amer. J. Pathol.* 46:929-943.

The pathogenesis of Sindbis virus encephalitis in suckling mice was studied by titration and histologic and fluorescent staining techniques. The first two methods yielded little information of the pathogenesis of infection, but reconstruction of cellular events during the incubation period was possible with immunofluorescent methods. After subcutaneous inoculation virus multiplied in striated, smooth, and cardiac muscle and in vascular endothelium. A high-titered plasma viremia developed rapidly. Virus spread to the CNS from the blood, and virus antigen was demonstrated in the endothelium of small cerebral vessels prior to its development in brain. Virus was disseminated rapidly within cerebral parenchyma,

infecting both neural and glial cells. The mechanisms of hematogenous spread of viruses to the CNS are discussed in relation to sites of extraneural growth, maintenance of viremia, modes of invasion of CNS from blood, and variations in cell infection and virus spread within the CNS.

5499

Karmysheva, V.Ya.; Gavrilovskaya, I.N.; Chumakov, M.P. 1965. Interaction of the Omsk hemorrhagic fever virus with cells of a susceptible tissue culture. Vop. Virusol. 10:557-563. In Russian.

Omsk hemorrhagic fever virus causes an acute infection with cytopathic effect in swine embryo kidney cell culture. In the cytoplasm of infected cells oxyphilous, RNA-containing inclusions and vacuoles are formed, the content and distribution of lipids are changed, and viral antigen is accumulated. Processes of karyopycnosis and karyorhexis are observed. The amount of the virus in the cultural fluid increases with the accumulation of RNA-containing material in the cell cytoplasm and the intensity of fluorescence of a specific antigen.

5500

Kundin, W.D. 1963. Studies on West Nile virus infection by means of fluorescent antibodies: II. Pathogenesis of West Nile virus infection in experimentally inoculated chicks. Arch. Ges. Virusforsch. 12:529-536.

In pathogenesis studies of West Nile virus in chicks inoculated subcutaneously, viral antigens were found predominantly in the gizzard and heart, in the smooth muscle layers of blood vessels and the digestive tract, and occasionally in the brain and the deep glands of the proventriculus. Antigens were demonstrable from the 2nd to the 8th days, at which time antibodies appeared. Except for blood and the brain, the appearance of infectious virus generally paralleled that of antigenicity. Neural involvement appeared to be minor.

5501

Kundin, W.D.; Liu, C. 1963. Effects of acetone, ultraviolet irradiation, and formalin on West Nile virus infectivity and immunofluorescent antigenicity. Proc. Soc. Exp. Biol. Med. 114:359-360.

Infectivity of West Nile virus in frozen sections was inactivated by UV light in a matter of minutes, but antigenicity persisted for several hours. Immersion of infected frozen sections in acetone inactivated 99 per cent of virus infectivity in minutes, yet antigenicity was not impaired for as long as 2 weeks at room temperature. In our hands fixation by formalin was unsatisfactory.

5502

Kundin, W.D.; Liu, C.; Hysell, P.; Hamachige, S. 1963. Studies on West Nile virus infection by means of fluorescent antibodies: I. Pathogenesis of West Nile virus infection in experimentally inoculated suckling mice. Arch. Ges. Virusforsch. 12:514-528.

The pathogenesis of West Nile virus in suckling mice was studied by infectivity titration of tissues and fluorescein-labeled antibody staining. When mice were inoculated intracerebrally, antigens were first detected in the central nervous system and in mesenchymal tissue. When mice were inoculated intraperitoneally, antigens were first detected in mesenchymal tissue and the plexus of Auerbach in the digestive tract. Additional antigens were soon found in the entire nervous system, the blood vessels, the smooth and skeletal muscle, and other connective tissues. Involvement of the major organs and lymph nodes was minimal until the last 2 days of infection. The rise of tissue infectivity titers generally paralleled the observed spread of viral antigens. Nervous tissue and mesenchymal tissue appeared to show equal susceptibility to primary viral invasion and growth. The possibility that the blood vessel wall is one of the primary sites of multiplication for West Nile virus is suggested.

5503

Kunz, C. 1964. The use of the immunofluorescent method and microphotometry for the differentiation of arboviruses. Virology 24:672-674.

The combined application of the immunofluorescent method and microphotometry will serve as a sensitive and valuable tool for the differentiation of closely related viruses. A Reichert microphotometer was used. Fluorescence in cells was measured. In most instances the brightest fluorescence was obtained in homologous systems.

5504

Kunz, C. 1965. On the usability of fluorescence serology and microphotometry for the differentiation of viruses of the tick-borne encephalitis (TBE) group. Zentralbl. Bakteriell. Parasitenk. Infektionskrankh. Hyg. Abt. I 196:4:416-437. In German.

An attempt was made to differentiate the following viruses of the tick-borne encephalitis (TBE) group in HeLa cells by FA: Central European encephalitis (early summer meningo-encephalitis) virus, Far Eastern Russian encephalitis virus, louping ill virus, Omsk hemorrhagic fever virus, Kyasanur Forest diseases virus, and Langat virus. The direct and the indirect FA was used. By the direct method virus-specific antigen in the course of the infection could only be demonstrated with the homologous conjugates in the cytoplasm. Distribution of antigen and the extent of the antigen aggregates showed characteristics that were not sufficiently marked for a clear differentiation of the viruses.

Heterologous conjugates gave no or weaker fluorescence than homologous conjugate. Human convalescent sera proved more specific than hyperimmune sera of mice. By means of a microphotometer it was possible to measure the intensity of the specific fluorescence and thereby obtain an objective measure for the degree of the serological cross-reaction. This permitted differentiation of the six virus types. The combined application of the direct immunofluorescent method and microphotometry represents a promising new way of differentiation of the antigenic structure of closely related viruses. Indirect FA did not prove suitable for determination of differences in antigenic structure of viruses of the TBE group.

5505

Kusano, N. 1964. Studies on the pathogenesis of Japanese encephalitis. *Acta Pathol. Jap.* 14:207-209.

Direct FA was used to study the distribution of virus antigen in infected adult and suckling mice. Neurotropism of this virus was confirmed. FA detected virus in four of eight samples from human brain autopsy specimens.

5506

Libikova, H.; Mayer, V.; Kozuch, O.; Rehacek, J.; Ernek, E.; Albrecht, P. 1964. Isolation from Ixodes persulcatus ticks of cytopathic agents (Kemerovo virus) differing from tick-borne encephalitis virus and some of their properties. *Acta Virol.* 8:289-301.

Agents highly cytopathic for, and forming plaques in, chick embryo cell (CEC) cultures and nonpathogenic for adult laboratory animals were isolated in newborn mice and CEC from adult Ixodes persulcatus ticks collected in the Kemerovo region in West Siberia. Together with strains isolated later from human beings, they were named Kemerovo virus. Incidence of these viruses in adult ticks was 3.2 per cent. The Romanovka and Kuchum strains of Kemerovo virus multiply, causing death of chick embryos 2 days after inoculation into the yolk sac. They do not agglutinate fowl, goose, or human O erythrocytes. Using sera from immunized guinea pigs and mice, it was confirmed that these viruses belonged to one group. By protection tests in mice their relationship to tick-borne, St. Louis and Japanese encephalitis, western and eastern equine encephalomyelitis, Sindbis, Colorado tick fever, pseudo-rabies, and lymphocytic choriomeningitis virus was excluded. Substances neutralizing the new virus were found in the sera from horses, cattle, birds, small mammals, and human beings in the respective localities. FA detected viral antigen in cell cytoplasm.

5507

Maguire, T.; Miles, J.A.R. 1965. The arbovirus carrier state in tissue cultures. *Arch. Ges. Virusforsch.* 15:457-474.

The methods employed for the establishment of latently infected arbovirus carrier cultures have been described. Once established the carrier cultures appeared to be similar to one another in their properties. These included the ability to resist superinfection with homologous and heterologous viruses, slower growth rates than uninfected cells, morphological similarity to uninfected cell populations, and low proportion of cells actually carrying virus. No evidence has been found of a lysogenic relationship similar to that occurring with temperate phages and bacteria. Evidence is presented that indicates that interferon is intimately associated with the maintenance of the carrier state, and that conditions that interfere with the level of interferon could result in an increase in CPE and virus output.

5508

McGavran, M.H. 1964. Electron microscopic and immunofluorescent observations in monkey livers and tissue culture cells infected with the Asibi strain of yellow fever virus. *Amer. J. Pathol.* 44:9a.

Samples of Macaca mulatta liver and hamster kidney tissue culture cells (HKL) were fixed in one per cent osmium tetroxide and frozen in isopentane at -70 C at various intervals following inoculation with yellow fever virus (Asibi strain). The osmium-fixed materials were processed in Epon 812, sectioned with diamond knives, stained with uranyl nitrate, and viewed with an RCA EMU-3F microscope. The frozen samples were cut at 4u in a cryostat and then FA stained with a conjugate of yellow fever - immune globulin. Specific immunofluorescence representing viral antigen was detected at 15 to 18 hours in the cytoplasm of the HKL cells. It eventually filled the entire cytoplasm. No specific fluorescence was found through 96 hours in the parenchymal cells of the monkey livers, well past the appearance of cytologic alterations. Electron micrographs showed the development of membrane-limited virus particles in the HKL cells, but not in the liver cells. Instead, by 96 hours the cytoplasm was filled by clusters of particles similar to ribosomes. These findings illustrated the differences between virus-cell interaction in vivo and in vitro. The particles seen in hepatic parenchymal cells may not be virus but rather a morphologic manifestation of altered cell metabolism. Complete article.

5509

McGavran, M.H.; White, J.D. 1964. Electron microscopic and immunofluorescent observations on monkey liver and tissue culture cells infected with the Asibi strain of yellow fever virus. *Amer. J. Pathol.* 45:501-517.

When hamster kidney cells in tissue culture are inoculated with the Asibi strain of yellow fever virus, specific viral antigen appears in the cytoplasm by 12 to 15 hours and gradually increases in amount. Ultrastructural examination of these cells shows characteristic virus particles that measure approximately 42 millimicrons. The liver cells of cynomolgus monkeys inoculated with yellow fever virus show a progressive loss of glycogen and a reorganization of the ribosomes into

clusters. Viral antigen is not demonstrated in these cells, and no structure characteristic of a virus particle is found there. It is hypothesized that these clusters of 20-millimicron particles having the appearance of ribosomes are polyribosomes and not virus.

5510

Nir, Y.; Beemer, A.; Goldwasser, R.A. 1965. West Nile virus infection in mice following exposure to a viral aerosol. *Brit. J. Exp. Pathol.* 46:443-449.

The dissemination of virus in selected tissues of mice at different time intervals after exposure to an aerosol of West Nile virus was studied by the simultaneous use of animal titration, FA, and histological techniques. No virus in significant amounts was found in the liver, spleen, kidneys, adrenals, cervical lymph nodes, and nasal mucosa during the first 4 days following infection, and no lesions were seen in these tissues at any time. Proliferation of virus in the lung was observed as soon as 24 hours after infection. FA staining was found in the cytoplasm of macrophages in this tissue. No pathological damage was seen on histological examination. Maximal multiplication of virus took place in the CNS in which occurred the only lesions detected by histopathological techniques. First signs of the presence of virus appeared in the olfactory bulbs before its appearance in the midbrain and the cerebellum. Following exposure of mice to an aerosol of West Nile virus, invasion of the CNS probably occurs through the olfactory pathway.

5511

Parfanovich, M.I.; Sokolov, N.N. 1965. Interaction of nucleic acids and antigens in tissue culture cells doubly infected with measles and tick-borne encephalitis viruses, as revealed by fluorescence microscopy. *Acta Virol.* 9:352-357.

After infecting sheep embryo kidney cell cultures with both tick-borne encephalitis (TBE) and measles viruses, both viruses multiplied, but the levels reached by either virus and the accumulation of their antigens depended on the sequence and intervals at which the two agents were inoculated. In mixed infected cultures, the cytopathic effect (CPE) characteristic of measles virus in the given cells was enhanced; however, there remained areas of cells without a CPE but showing an accumulation of TBE virus antigen and RNA-containing material. Distribution of viral antigens and of nucleic acids differed from that found in cells infected with either virus alone. Measles virus antigen and the substance showing green fluorescence after staining with acridine orange occurred mostly in the cytoplasm and not as agglomerates in the nuclei, as with single measles virus infection. Antigen of TBE virus and the RNA-containing substance occurred not only in the cytoplasm, as with single TBE virus infection, but also as agglomerates in the nuclei.

5512

Pirosky, I.; Teyssie, A.; Ayerra de Holstein, B.; Rodriguez, A.; D'Empaire, M. 1961. Hemorrhagic viruses of northwestern Buenos Aires: VI. Visualization of the specific viral antigen by means of the fluorescent antibodies technique. Acta Krausi Cuad. Inst. Nac. Micro. Biol. Buenos Aires 3:64-65. In Spanish.

The brains of suckling mice were inoculated with the R5 strain of the virus. At the 11th day the brains were removed, when the mice showed the typical picture of the disease. FA was applied to brain sections. Results showed a large amount of fluorescent material corresponding to specific viral antigen particles. BA-46-39753.

5513

Fogodina, V.V.; Shi-Tsze, K. 1964. Studies of correlation between pathogenicity of tick-borne encephalitis viruses for animals and peculiarities of their growth in the organism: I. Correlation between viscerotropy and pathogenicity of virus strains with peripheral inoculation. Vop. Virusol. 9:682-690. In Russian.

Two strains of tick-borne eastern encephalitis virus were studied with respect to their peculiarities of multiplication in mice. The strains differed in virulence when introduced peripherally, although they possessed equally high pathogenicity for intracerebrally infected mice, hamsters, pigs, and monkeys. Differences in the peripheral activity were assessed clinically by subcutaneous titration in mice, virologically by content of infectious virus in organs, and by immunofluorescence for detection of virus antigen in tissue. The Xa6-17 strain, highly virulent when inoculated peripherally, possessed a marked viscerotropy. It grew readily in the atrium of infection in various extra-neural tissues and then in the central nervous system. Infection was accompanied by intensive viremia. The Fateev strain, weakly virulent with peripheral inoculation, grew poorly, mainly in the atrium of infection; occasionally it could be revealed in some internal organs, but the viremia was of low degree. To reach the central nervous system an infecting dose of the Fateev strain 100 to 10,000 times greater than that of the Xa6-17 strain was necessary. The low viscerotropy of the Fateev strain was observed with subcutaneous inoculation of different doses and did not depend on the sensitivity of a method of assay.

5514

Pogodina, V.V.; Shi-Tsze, K. 1965. Studies on correlation between pathogenicity of tick-borne encephalitis viruses for animals and peculiarities of their growth in the organism: II. Vop. Virusol. 10:30-36. In Russian.

By the fluorescent antibody method a marked viscerotropy and neurotropy of the Malaya Langat virus, TR-21 strain, could be established despite a weak pathogenicity of the virus for various animals inoculated intracerebrally or intravenously. The peculiarity of the pathogenesis of infection induced by the TR-21 strain consisted of the discrepancy between infection signs in cells, by FA stain, and the content of virus infective for mice. In many experiments carried out with mice, hamsters, and pigs, the virus antigen, although of very low infectious titer, was demonstrable in many nerve cells. It is suggested that a low virulence of some viruses of this group, with peripheral inoculation, may be due to a weak viscerotropy of a strain or to the formation of noninfectious virus.

5515

Shi-Gie, H. [Shi-Tsze, K.]; Pogodina, V.V. 1964. Use of fluorescent antibody technique in studies on Far East tick-borne encephalitis in mice, hamsters, and pigs. Acta Virol. 8:22-29.

Multiplication of the Far East strains of tick-borne encephalitis virus (RSSE virus) in the central nervous system, subcutis at the site of inoculation, lymph nodes, intestine, and spleen was demonstrated by the fluorescent antibody technique. This viscerotropism, including the intestine, was inherent to various strains of the RSSE virus and occurred after subcutaneous or alimentary infection of mice, hamsters, and piglets. Two phases of virus multiplication were demonstrated on peripheral inoculation, a visceral and neuronal phase. The direct fluorescent antibody method proved sufficiently sensitive for pathogenetic studies on tick-borne encephalitis in various animals.

5516

Shi-Tsze, K.; Pogodina, V.V. 1965. Use of the fluorescent antibody method for study of the course of infection in the immune organism in tick-borne encephalitis. J. Hyg. Epidemiol. Microbiol. Immunol. 9:346-355.

FA was used to study the characteristics of the pathogenesis of tick-borne encephalitis in the organism of immunized mice with different degrees of humoral immunity. The phase of multiplication of TE virus in the visceral organs (the visceral phase) is primarily inhibited

in the passively immunized organism. The tick-borne encephalitis virus multiplies on a small scale at the site of inoculation in passively immunized mice and attacks the central nervous system to varying degrees, according to the degree of humoral immunity and viremia. Direct FA can be used for study of the pathogenesis of infection in the immune organism.

5517

Sokolov, N.N.; Parfanovich, M.I.; Mekler, L.B. 1963. On the nature of tick-borne encephalitis virus: I. A comparative study of nucleic acids and specific antigen in sheep embryo kidney cell cultures infected with tick-borne encephalitis virus by fluorescence microscopy. *Acta Virol.* 7:209-216.

During the reproduction of tick-borne encephalitis virus in sheep embryo kidney cell cultures, acridine orange staining revealed a redistribution of the orange-red RNA-containing material, which concentrated in the perinuclear area and, at final stages of infection, filled the whole cytoplasm. When studying the reproduction of tick-borne encephalitis virus in sheep embryo cells by the fluorescent antibody method, fluorescence was first observed in the nucleoli and then as a dotted line around the nuclei; thereafter the specific antigen concentrated mainly in the perinuclear area. At late stages of infection the fluorescence extended throughout the cytoplasm to the cellular membrane. The parallelism between the distribution of RNA-containing material, the localization of specific antigen in the cytoplasm, and the appearance of virus in the medium in sheep embryo kidney cell cultures infected with tick-borne encephalitis virus points to a possible relationship between the RNA substrate of the cytoplasm and the tick-borne encephalitis virus antigen, or to a possible participation of the RNA-containing material in virus synthesis.

5518

Sokolov, N.N.; Parfanovich, M.I.; Mekler, L.B. 1963. On the nature of tick-borne encephalitis virus: II. A comparative study of nucleic acids and specific antigen in cells from brains of white mice infected with tick-borne encephalitis virus by fluorescence microscopy. *Acta Virol.* 7:217-224.

In the course of experimental tick-borne encephalitis in white mice a parallelism between the distribution of RNA-containing material and specific antigen could be demonstrated in the neurons of the cornu Ammonis and the Purkinje cells of the cerebellum, which offers suggestive evidence of a participation of RNA in the synthesis of tick-borne encephalitis virus antigen. The coincidence found between the production and distribution of RNA-containing material and of specific antigen in the course of tick-borne encephalitis infection of white mice demonstrated certain peculiarities of virus interaction with cells of the central nervous system, namely a specific fluorescent RNA-containing material in the nucleoli, its penetration through the nuclear membrane and concentration in the cytoplasm, and release from the cells.

5519

Southam, C.M.; Shipkey, F.H.; Babcock, V.I.; Bailey, R.; Erlandson, R.A. 1963. Growth of two arbor viruses in tissue cultures. *Federation Proc.* 22:2373:557.

Hep-2 cells in monolayer culture were infected with West Nile or Guaroa virus or kept as controls. At daily intervals for 4 days, by which time cytopathology was severe, replicate cultures were studied by intracerebral inoculation of mice for infectivity, by fluorescent antibody for distribution of virus antigen, by acridine orange for cytochemical evidence of changes in nucleic acids, and by electron microscopy for morphology and distribution of particles. Both viruses showed maximum infectivity by the 2nd day. At 1 and 2 days there was increased cytoplasmic RNA. Frequency of FA-positive cells increased steadily to about 50 per cent by 4 days. West Nile was diffusely disseminated through cytoplasm, but Guaroa occurred in cytoplasmic granules. By day 4, electron microscopy revealed virus particles in numerous cells. West Nile occurred in viroplasts or diffusely through cytoplasm in association with endoplasmic reticulum. Guaroa occurred as extracellular clusters and in cytoplasmic vesicles. There was no convincing evidence of intranuclear virus by either electron microscopy or FA. Complete article.

5520

Southam, C.M.; Shipkey, F.H.; Babcock, V.I.; Bailey, R.; Erlandson, R.A. 1964. Virus biographies: I. Growth of West Nile and Guaroa viruses in tissue culture. *J. Bacteriol.* 88:187-199.

Hep-2 cells were inoculated with Guaroa or West Nile viruses. At daily intervals cultures were studied by light microscopy, intracerebral inoculation of mice, acridine orange stain, FA, and electron microscopy. Guaroa virus infectivity increased progressively and caused definite cytolysis by day 3. Cytoplasmic RNA staining was increased by 24 hours, and by 48 hours the RNA formed globular masses, particularly in degenerating cells. Viral antigen was seen on day 1, and increased progressively to form numerous sharply outlined particles in cytoplasm concentrated at the cell membrane. Virus particles were ellipsoids with a dense nucleoid and a single membrane approximately 70 by 90 mu, which appeared to form at the cell membrane. West Nile virus infectivity increased sharply between 1 and 2 days, but caused little cytolysis. Cytoplasmic RNA staining increased progressively, usually forming a large juxtannuclear mass in each affected cell. Viral antigen was not detected on day 1 but later formed a crescent or a single diffuse mass adjacent to the nucleus. Virus particles were spheres approximately 30 mu in diameter, with a dense nucleoid and a single membrane. They formed in granular foci in the cytoplasm and then filled channels of the endoplasmic reticulum. No significant nuclear changes were observed.

5521

Takeda, H.; Yamada, M.-A.; Aoyama, Y. 1965. Demonstration of RNA synthesis caused by Japanese encephalitis virus infection in PS(Y-15) cells with the aid of chromomycin A-3. Jap. J. Med. Sci. Biol. 18:111-120.

PS(Y-15) cells, a porcine kidney stable cell line, were infected with Japanese encephalitis virus (JEV), a member of RNA-containing viruses. Immediately after virus adsorption, the cells were treated with chromomycin A-3 in a concentration sufficient to suppress cellular RNA synthesis. The synthesis of viral particles and the production of viral antigen in chromomycin-treated cells were similar to those in nontreated controls in respect to the rate as well as the time course. The results suggest that chromomycin A-3 does not inhibit RNA synthesis by JEV infection.

5522

Taylor, J. 1965. Studies on the mechanism of action of interferon: I. Interferon action and RNA synthesis in chick embryo fibroblasts infected with Semliki Forest virus. Virology 25:340-349.

Semliki Forest virus (SFV) growing in chick embryo fibroblasts (CEF) has been used in a study of the action of interferon in inhibiting virus growth. Growth of virus is inhibited in cells pre-exposed to interferon. CEF cells infected with SFV produce interferon, but inasmuch as it is produced late in the growth cycle, it does not appear to have a marked inhibitory effect on the first cycle of growth. The RNA synthesis in SFV-infected cells, which is resistant to actinomycin, can be completely inhibited by pretreatment of cells with purified preparations of chick interferon. However, interferon does not inhibit virus growth in cells previously treated with actinomycin. Infection of CEF cells with SFV at a multiplicity of 10:1 does not result in a specific cutoff of host RNA synthesis during the latent period, but synthesis of host RNA decreases when the synthesis of viral RNA begins. Cell infection was demonstrated by indirect FA.

5523

Vainio, T.; Saxen, L.; Toivonen, S. 1963. Viral susceptibility and embryonic differentiation: 2. Immunofluorescence studies of viral infection in the developing mouse kidney in vitro. Acta Pathol. Microbiol. Scand. 58:205-211.

The distribution of vesicular stomatitis virus (VSV) antigen in fetal mouse kidney in vitro closely corresponds to that of the histologically observed viral lesions. Furthermore, this study supports our earlier suggestion of certain differences in the tissue competence (susceptibility) toward the two viruses VSV and polyoma virus employed in our

studies so far. Attention was focused on changes during tubulogenesis, where three morphologically distinct stages can be seen: Undifferentiated metanephrogenic mesenchyme leads to mesenchymal condensations, which are found in epithelial tubules. The mesenchyme seems to support the replication of both viruses, but renal tubules appear to be resistant. Mesenchymal condensations, which represent the first detectable step in tubulogenesis, resist polyoma virus but are highly susceptible to VSV and support its replication. The induction process interferes with the susceptibility of a given tissue to a particular virus. However, the tissue can remain susceptible to some other viruses. Processes relevant to differentiation, such as metabolic changes in the differentiating cells, have a bearing on viral susceptibility.

5524

Wallnerova, Z.; Albrecht, P. 1964. Detection of Tahyna virus in tissue cultures by the fluorescent antibody technique. *Acta Virol.* 8:474.

The FA technique was used to detect antigen of a member of the California encephalitis virus complex. Direct FA was superior to indirect FA.

5525

Zhdanov, V.M.; Stakhanova, V.M.; Yershov, F.I. 1964. Polycistronic composition of viral RNA. *Vop. Virusol.* 9:564-569. In Russian.

Different sensitivity of individual loci of genetic material of bacterial and viral nucleic acids to outside agents has been shown. This was found with myxoviruses exposed to heating and ultraviolet radiation. An alteration in RNA metabolism caused by partly inactivated virus, by RNA preparation isolated from myxovirus infected cells, and by S antigen of the same viruses indicates that these preparations are not inert. Heating of virus and phenol extraction of RNA result in incomplete inactivation of genetic information contained in viral RNA. Certain loci of the latter responsible for RNA synthesis and, apparently, for early protein synthesis remain intact. A linear rise in metabolism of an amino acid, methionine-S85, observed in infection with inactivated virus is indicative of increased synthesis of a protein material. A sharp decrease in methionine incorporation in actinomycin D - treated cells suggests the presence of interferon produced by tissues in response to introduction of inactivated virus. Experiments with VEE have shown that newly formed mature virus can be detected in 2.5 to 3 hours. This time is equal to the latent phase of growth. As the release of virus after maturation takes only 20 seconds, one may suppose that this time is necessary mainly for synthesis of viral proteins and assembly of virions. Maturation of virus lasted for 1 or 2 hours after introduction of the antibiotic.

II. POXVIRUS

5526

Appleyard, G.; Westwood, J.C.N. 1964. The growth of rabbitpox virus in tissue culture. *J. Gen. Microbiol.* 37:391-401.

The growth of rabbitpox virus has been studied in monolayer cultures of HeLa cells. At 36 C, virus was adsorbed to cells at about one-tenth of the theoretical collision rate. The adsorbed virus penetrated the cells exponentially, the time for half penetration being about one hour. New infective virus began to appear at 5 hours, and the final yield at 24 hours was about 100 pfu per cell. The synthesis of viral antigen began 1.5 hours after infection of the cultures. This was one hour before the earliest appearance of viral DNA. When virus growth was complete, over 20 separate antigens could sometimes be detected in extracts of infected cells. The yield of antigens was not reduced when virus multiplication was completely inhibited by bromodeoxyuridine. The synthesis of viral antigens was directed by the DNA of the infecting virus, and not by the DNA formed during virus growth.

5527

Bals, M.; Caruntu, F. 1965. Contribution to the rapid diagnosis by immunofluorescence of infection and immunity in variola-vaccinia. *Rev. Roumaine Inframicrobiol.* 2:3:187-193.

Description is given of a direct FA technique with fluorescent anti-vaccinia human serum used for detecting antigen, and an indirect technique for serologic titration of human serum antibodies. A fluorescent anti-human serum and vaccine substrate were employed.

5528

Baratawidjaja, R.K.; Morrissey, L.P.; Labzoffsky, N.A. 1965. Demonstration of vaccinia, lymphocytic choriomeningitis and rabies viruses in the leucocytes of experimentally infected animals. *Arch. Ges. Virusforsch.* 17:271-279.

During the stage of viremia, viral antigen was demonstrable by means of immunofluorescence in the leukocytes of peripheral blood of experimental animals infected with vaccinia, lymphocytic choriomeningitis, and rabies viruses. Specificity of the fluorescence observed was confirmed in each case by re-isolation of the virus from washed leukocytes by animal or tissue culture inoculation and the identity of re-isolated virus was established by neutralization or immunofluorescence techniques. Possibility of employing FA for early diagnosis of viral infections is discussed.

5529

Bueno, F.L. 1964. A study of vaccinia virus. Rev. Sanid. Hig. Publica 38:225-243. In Spanish.

Staining of cells infected with the virus of antivariola vaccine demonstrated inclusion bodies made up of DNA. Immunofluorescence revealed that virus antigen was present. Location of the inclusion bodies varied.

5530

Carter, G.B. 1965. The rapid detection, titration, and differentiation of variola and vaccinia viruses by a fluorescent antibody - cover slip cell monolayer system. Virology 25:659-662.

Observations of sequential changes within infected cells were generally in agreement with the results of other workers. Variola and vaccinia were differentiated by growth characters within the cells and incubation temperature results. FA has value in rapid detection of poxviruses and may be diagnostically useful.

5531

Easterbrook, K.B.; Davern, C.I. 1963. The effect of 5-bromodeoxyuridine on the multiplication of vaccinia virus. Virology 19:509-520.

In the presence of 5-bromodeoxyuridine (BDU) vaccinia-infected HeLa or KB cell cultures develop cytoplasmic foci that stain specifically with fluorescein-coupled rabbit antiserum to vaccinia. These foci are able to incorporate tritiated thymidine on removal of the BDU. The cells yield large quantities of noninfectious, malformed virus particles. Kinetic studies on the establishment of this inhibition, and density gradient analyses of the DNA from BDU-vaccinia, indicate that these abnormalities are a consequence of the incorporation of BDU into the virus DNA. Various poxviruses appear to differ in their susceptibility to BDU inhibition.

5532

Gurvich, E.B.; Roihel, V.M. 1965. Use of the fluorescent antibody technique in the detection and differential diagnosis of smallpox. Acta Virol. 9:165-171.

Studies on variola, alastrim, vaccinia, cowpox, varicella, herpes zoster, and herpes simplex viruses were carried out by the direct fluorescent antibody technique. Antigen of variola virus could be detected 6 hours after infection of human embryo cell cultures with 1,000 tissue culture mean infective doses of the virus. With smaller doses of virus, the time periods for virus detection increased. With 1 and 0.5 mean

infective doses, the viral antigen was detected in a part of the inoculated cultures after 3 days. Some peculiarities in the intracellular distribution of fluorescence caused by the presence of different viral antigens were observed. An antigenic relationship between the varicella and herpes viruses was demonstrated. Cross-reactions with homologous and heterologous globulins revealed the possibility of serological identification of viruses of the pox group as well as of their differentiation from the varicella and herpes viruses.

5533

Hahon, N. 1965. Assay of variola virus by the fluorescent cell-counting technique. *Appl. Microbiol.* 13:865-871.

A quantitative assay for infective variola virus particles was developed that is based on the enumeration of cells containing fluorescent viral antigen after infection of McCoy cell monolayers. Direct FA was employed to stain cells. The efficiency of virus adsorption was markedly enhanced by centrifugation of virus inoculum onto McCoy cell monolayers at 500 x g for 15 minutes. By this procedure, a proportionality was obtained between the number of fluorescent cells and volume of inoculum. Observations on the sequential development of viral antigen within cells and counts of fluorescent cells showed that the optimal time for enumerating fluorescent cells was after an incubation period of 16 to 20 hours. A linear function existed between virus concentration and cell-infecting units. Fluorescent cells were distributed randomly in infected cover slip cell monolayers. The assay was demonstrated to be highly sensitive, precise, and reproducible.

5534

Huang, J.S.; Bang, F.B. 1964. The susceptibility of chick embryo skin organ cultures to influenza virus following excess vitamin A. *J. Exp. Med.* 120:129-148.

The conversion of chick embryonic epidermis to mucous epithelium by excess vitamin A in organ culture was accompanied by a corresponding change of susceptibility to influenza and vaccinia viruses. Untreated epidermis of 10- to 12-day chick embryos supported the growth of influenza virus in organ cultures and a maximum infectivity titer was reached 2 to 3 days after infection. At the same time, the epidermis showed squamous keratinization, beginning about the 4th day of cultivation. Addition of excess vitamin A to the skin organ culture induced the following changes: mucous metaplasia of the epidermis, increase in the daily and maximum yield of influenza virus, and decrease in the production of vaccinia virus. The maximum yield of both viruses remained unchanged if vitamin A was introduced at the time of virus inoculation. The yield of influenza virus system was proportionally related to the concentration

of vitamin A added 4 or more days before inoculation of virus. Increasing doses of vitamin A had no effect on the short-term growth of influenza virus in tissue cultures of chorioallantoic membrane. The change of virus specificity is not due to excess vitamin A, but appears related to the change of differentiation produced in the organ culture system.

5535

Kato, S. 1964. Diagnosis of smallpox and cowpox by fluorescent antibody technique. *Clinic-Around (Osaka)* 13:1239-1247. In Japanese.

Fluorescent antibody technique is very useful and highly specific for the diagnosis of smallpox and cowpox. However, it is difficult to differentiate these two, or other viruses belonging to this group. Differentiation between smallpox and cowpox virus is feasible because cells infected by the latter form Type A inclusion bodies, which appears as a peculiar ring form fluorescence when stained with rabbit anti-serum. Specimens which can be FA stained include virus infected tissue culture cells, rabbit corneal epithelial cells (Paul reaction), and the smears of the eruption.

5536

Keller, R.; Braunstein, H. 1965. Differential response of lysosomes in vaccinia-infected cell cultures. *Bacteriol. Proc.* V62:107.

Monolayer cultures of serially propagated cell lines infected with a strain of dermal vaccinia, adjusted to initiate discrete foci of infection, have demonstrated two distinct patterns of cytopathic responses. Within two of the cell lines, HeLa and Chang conjunctiva, the infective foci developed large multinucleated giant cells. On the other hand, infected cells of Hep-2, KB, LLCMK₂, the diploid cell strain WI-38, and the S-3 clone of HeLa demonstrated only minimal cell fusion. Intense development of the two lysosomal enzymes was demonstrated in the form of minute spherical particles around progressively enlarging eosinophilic inclusion bodies forming within the multinucleated giant cells; the surrounding cytoplasm of the structure did not demonstrate similar particles. These inclusions, which manifested high concentrations of viral antigenic material by the direct fluorescent antibody technique, continued to demonstrate high concentrations of enzymatic activity even after 3 days of incubation. The lysosomal complement of the fused cells had aggregated around the inclusion body, and the contents of the lysosomal units were not released. Within cell lines not forming giant cells, individual infected cells manifested a progressive loss of lysosomal enzymes as compared with uninfected surrounding cells. Variations in the disruption of lysosomes and the release of hydrolytic enzymes may influence the cytopathic response of different cell populations infected with a single strain of vaccinia virus.

5537

Kirsh, D.; Kissling, R. 1963. The use of immunofluorescence in the rapid presumptive diagnosis of variola. Bull. WHO 29:126-128.

Use of the fluorescent antibody technique, along with history and clinical findings, would materially aid differential diagnosis of disease problems of this kind.

5538

Kishida, T.; Kawamata, J.; Miyamoto, H.; Kato, S. 1965. Inhibitory effect of interferon on the multiplication of Shope fibroma virus in vitro. Biken J. 8:55-61.

Interferon prepared from cells infected with vaccinia virus inhibits not only tumor formation by SFV in rabbits but also the formation of inclusion bodies in vitro, indicating its eventual inhibition of SFV multiplication.

5539

Kratchko, A.; Netter, R.; Thivolet, J. 1964. A rapid method for demonstration of vaccinia virus by means of direct immunofluorescence. Ann. Inst. Pasteur Paris 107:184-191.

The authors have adapted the direct immunofluorescence technique to identification of vaccinia virus after 16 hours of culture on T cells of pathological materials. The method was applied to the demonstration of the virus in a certain number of human and animal materials; it proved sensitive and specific. Its reliability in routine practice is compared with that of other techniques, and its use in the diagnosis of the disease is proposed.

5540

Loh, P.C.; Payne, F.E. 1965. Effect of p-fluorophenylalanine, on the synthesis of vaccinia virus. Virology 25:560-574.

The effect of the amino acid analog p-fluorophenylalanine (FPA) on vaccinia virus replication in HeLa cells was investigated. By varying the dose of FPA or the time after infection at which the analog was added to cultures, it was possible to inhibit differentially the synthesis of viral components in vaccinia-infected cells. When sufficient FPA was added to cultures at the time of infection, it inhibited the synthesis of viral DNA, nucleoprotein antigen, infectious virus, and hemagglutinin. The inhibition of synthesis of heat-labile heat-stable antigen (LS) under all conditions studied was only partial. FPA added to cultures at 6 or more hours postinfection did not inhibit production of viral antigens or infectious virus. Direct FA was used to stain LS and nucleoprotein antigens selectively.

5541

Lycke, E.; Ahren, C.; Stenborg, R.; Bernler, G.; Spetz, S. 1963. A case of intrauterine vaccinia. *Acta Pathol. Microbiol. Scand.* 57:287-294.

A woman of 29 was vaccinated in the 23rd week of pregnancy. Five weeks later she bore an immature girl with severe skin lesions, who died on the 8th day. Vaccinia virus was isolated from placenta and vaccinia antigen was demonstrated in placenta and skin samples by fluorescent antibody technique. Cytoplasmic inclusion bodies positive to Feulgen stain were demonstrated in epidermal and stromal cells surrounding the skin and placental lesions. Involvement of placenta and internal organs indicates a hematogenic virus transmission. Whether transmission of virus may also occur by the amniotic fluid remains to be proved. Intrauterine vaccinia infection is a rare complication to vaccination. It might be advisable to avoid vaccination of pregnant women.

5542

Mastyukova, Yu.N.; Sumarokov, A.A.; Sarayeva, N.T.; Gutkina, A.V.; Kulikov, Yu.M. 1963. On the mechanism of smallpox immunity: I. The role of leukocytes in smallpox immunity experiments in vivo. *Vop. Virusol.* 8:492-498. In Russian.

In vaccinia-immunized and nonimmunized rabbits peritoneal exudates caused by the introduction of a nonspecific irritant into the peritoneal cavity were similar as to their cell content; more than 70 per cent of the cells were special granulocytes. After an intraperitoneal inoculation of neurovaccine the character of cellular response was quite different: in immune animals mononuclear cells prevailed in the inflammation focus. In nonimmune animals only an insignificant polyblastic reaction was noted. Vaccinia virus was quickly phagocytized by leukocytes in immunized and nonimmunized rabbits. However, the virus multiplied only in leukocytes of nonimmunized animals. Failure of virus to multiply in leukocytes of immune animals was due to the inhibitory effect of specific antibody. Granulocytes that phagocytized virus under given experimental conditions were not able to disintegrate it. FA was used to demonstrate viral antigen in leukocytes.

5543

Murray, H.G.S. 1963. The diagnosis of smallpox by immunofluorescence. *Lancet* 1:847-848.

A method is described for the rapid diagnosis of smallpox by immunofluorescence. Several specimens from patients with smallpox were examined by this method; all gave positive results. Specimens from patients with chickenpox, examined at the same time, were negative.

5544

Nicoli, J.; Jolibois, C.; Bordas, J.; Demarchi, J. 1964. Poxvirus soluble antigens: II. Rapid diagnosis of small-pox; studies on agar precipitation and immunofluorescence test. *Ann. Inst. Pasteur* 107:453-457. In French.

Studies were done on the technique of rapid diagnosis by means of agar gel double diffusion precipitation and FA. The demonstration of small-pox antigen by means of agar double diffusion should be adopted in the epidemiological diagnosis because of the simplicity of the method.

5545

Nielsen, G. 1963. The action of p-fluorophenylalanine on the reproduction of vaccinia in HeLa cells. *Arch. Ges. Virusforsch.* 13:559-576. In German.

The reproduction of vaccinia virus was studied after exposure of HeLa cells to p-fluorophenylalanine (FPA) at various periods before and after inoculation, by determinations of infectious virus, by morphologic studies of inclusion bodies and by FA. When FPA was added 1 hour before or within 8 hours after inoculation with high multiplicity, there was no rise of infectivity or increase of detectable viral material inside the cells. FPA, given 12 hours and later did not interfere anymore. The effect of the inhibitor was reversible at various stages either by removing it from the medium or by adding phenylalanine. FPA did not influence the infectious virus or its incorporation into the cells. The formation of inclusion bodies and of viral antigen coincide with the rise of infectivity. After longer exposure to FPA the morphology of infected and normal HeLa cells was disturbed.

5546

Noyes, W.F. 1965. Observations on two pox-tumor viruses. *Virology* 25:666-669.

Yaba virus and molluscum contagiosum virus were studied. Electron microscopic comparisons were made. Direct FA was used to study Yaba virus infection of Cercopithecus monkey kidney primary tissue cultures. Localization was cytoplasmic.

5547

Peterson, O.P.; Kozlova, I.A.; Melnikova, L.A.; Sivoshinsky, D.S. 1964. Interaction of vaccinia virus with tissue culture cells. *Vop. Virusol.* 9:154-158. In Russian.

The development of vaccinia virus in chick embryo fibroblast cultures was studied by autoradiography and fluorescent antibody. The first reaction to the infection of the cell with virus was noted in the cell nucleus.

Three or four hours after infection the synthesis of the viral DNA was observed in the cytoplasm. Virus antigen was demonstrable still later. Autoradiography affords a promising method for studying the virus-cell interaction; with the application of tritiated thymidine the synthesis of viral DNA can be followed at early stages of the cell infection.

5548

Raskin, J. 1963. Molluscum contagiosum: Tissue culture and serologic study. Arch. Dermatol. 87:552-559.

A cytopathogenic effect was observed when molluscum contagiosum suspensions from 12 patients were passed into monolayer tissue cultures of monkey kidney, primary human amnion, and the F1 line of human amnion cells. Cytopathogenicity was associated with giant cell production as well as individual cell changes. Acidophilic, deoxyribonucleic acid-containing cytoplasmic inclusion bodies were identified in both acridine orange and Giemsa-stained preparations. Antibodies demonstrated by FA reacted specifically with the inclusion bodies. An attempt to infect a human being with virus-containing tissue culture material did not result in the production of overt disease.

5549

Roberts, J.A. 1963. Histopathogenesis of mousepox: III. Ectromelia virulence. Brit. J. Exp. Pathol. 44:465-472.

The difference between a virulent and an avirulent strain of ectromelia virus has been analyzed histologically by the fluorescent antibody technique. When inoculated intravenously the avirulent strain has difficulty in successfully infecting Kupffer cells, and when they are infected, Kupffer cells yield fewer infective particles of the avirulent virus strain. Up to 100 potentially infective particles of the avirulent strain of virus may be inoculated intravenously without inducing a detectable serum response. Once established in parenchymal cells, the viruses grow with equal facility.

5550

Roberts, J.A. 1964. Growth of virulent and attenuated ectromelia virus in cultured macrophages from normal and ectromelia-immune mice. J. Immunol. 92:837-842.

Growth of ectromelia virus in cultured mouse peritoneal macrophages is described. Macrophages in vitro are more susceptible to infection by a virulent than by an attenuated strain of ectromelia virus. Macrophages from susceptible and resistant strains of mouse are equally

susceptible to infection in vitro with virulent ectromelia virus. Macrophages from immune mice are more readily infected than are normal macrophages. This effect is attributed to the greater phagocytic activity of the macrophages from immune mice. Even in the presence of immune serum, macrophages from immune mice are more readily infected than are macrophages from normal mice.

5551

Soloviev, V.D.; Mastjukova, Yu.N.; Sumarokov, A.A.; Sarayeva, N.T.; Gutkina, A.V. 1965. Mechanism of antismallpox immunity: VI. Role of antigen in phenomena of specific insusceptibility. Vop. Virusol. 10:583-589. In Russian.

Studies in regularities of the immunologic process occurring on introduction of vaccina virus to rabbits have shown that the development of postvaccinal immunity is connected with alterations in lymphoid tissue cells such as activation of enzymatic systems and increase of mitotic and proliferative activity of reticular cells accompanied by a number of cell transformations. Specific antibodies are formed, and cell resistance rises. Lymphoid tissue cells exposed to antigen undergo deep biochemical, morphological, and functional changes. There is a correlation between the content of antigen in the lymphoid tissue and the extent of changes induced. Localization of these changes is dependent on the method of immunization and size of virus inoculum. The durability of immunity depends on the involvement of the organs rich in lymphoid tissue. For the development of a durable, complete postvaccinal immunity a generalized response of lymphoid organs is necessary. It results from dissemination of the virus in the body. The resistance of lymphoid tissue cells after immunization is not due to the failure in adsorption and penetration of virus into cells, but to the acquisition by cells of the ability to render infectious virus harmless.

5552

Sambrook, J.F.; McClain, M.E.; Easterbrook, K.B.; McAuslan, B.R. 1965. A mutant of rabbit poxvirus defective at different stages of its multiplication in three cell types. Virology 26:738-745.

A mutant of rabbit poxvirus (RPu6/2) fails to multiply in PK-2a cells, L cells, or HeLa cells, although multiplication in chick embryo fibroblasts is normal. The stage at which multiplication is blocked is different in each of the three nonpermissive cell types, as judged by tests for synthesis of viral DNA and viral proteins and by electron microscopy. Although the mutant uncoats normally in all cell types, it reactivates heated virus with very low efficiency in PK-2a cells. Hypotheses to account for these findings are discussed. Direct FA staining was used to visualize viral antigen in cells.

5553

Slotnick, V.B.; Rosanoff, E.I. 1963. Localization of varicella virus in tissue culture. *Virology* 19:589-592.

Varicella virus infection was followed in tissue culture by indirect FA. Varicella antigen, first detectable by immunofluorescence in the cytoplasm at the border of the nucleus, gradually accumulates in the perinuclear region and finally concentrates in the cytoplasm. Nuclear fluorescence was not observed before 72 hours. The cytoplasmic strains may play some role in the transfer of virus from cell to cell, but they are not unique for varicella virus - infected cells, as they also have been observed in cells infected with measles and with herpes virus.

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Sokolov, N.N.; Parfanovich, M.I. 1964. Character of the accumulation and localization of specific antigen and nucleic acids in the course of vaccinia virus infection of tissue culture as revealed by fluorescence microscopy. *Acta Virol.* 8:30-37.

In vaccinia virus - infected human embryo skin-muscle (HESM) cells stained with acridine orange, redistribution and accumulation of DNA-containing material was observed 6 hours after inoculation in the nuclei, cytoplasm, and cytoplasmic inclusions. Starting from the 8th hour after inoculation of the cells with vaccinia virus, specific antigen could be demonstrated by FA in the nucleoli, nuclear aggregates, in the perinuclear area of the cytoplasm, and in cytoplasmic inclusions. DNA-containing material filled cells in the direction from the periphery to the center. Formed inclusions were either homogeneously filled with the green DNA-containing material or contained RNA granules in the central part. Specific antigen also filled the inclusion. At late stages of infection the inclusions were either completely filled with specific antigen or contained negative areas in the center. Starting from the 12th hour after inoculation, symplasts were regularly formed. These multinucleate giant cells originated by fragmentation of mother nuclei into numerous daughter nuclei. In the symplasts there was a more intense accumulation of DNA-containing material, specific antigen, and cytoplasmic inclusions as compared with uninucleate cells.

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Tagaya, I.; Kitamura, T.; Sano, Y. 1963. Cytotoxic effect of active and inactivated dermovaccinia virus. Jap. J. Med. Sci. Biol. 16:277-294.

Cytotoxic effect (CTE) of dermovaccinia virus on Earle's L cell was investigated employing nigrosin staining as a measure of quantitative description of CTE. The cell agglutination and change in stainability of cells with nigrosin could be separated from each other in the time course of their development. The experimental data suggested that CTE as demonstrated by the nigrosin staining was manifested by some interaction of cells with adsorbed virus, which took place during several hours after adsorption. CTE analogous to that caused by active virus was also demonstrated by UV-inactivated virus. Inactivation with heat, periodate, and a bacterial protease destroyed this activity. FA was used to detect viral antigen in cells.

5556

Zhdanov, V.M.; Azadova, N.B.; Bukrinskaya, A.G.; Graf, I.A.; Zhantiyeva, Ye.M.; Klimenko, S.M.; Kozlova, I.A.; Konoshch, O.V.; Mekler, L.B.; Mel'nikova, L.A.; Naumova, V.K.; Peterson, O.P.; Sivoshinskiy, D.S.; Stakhanova, V.M.; Stefanov, S.B. 1963. Study of intracellular synthesis of viral components. Vestn. Akad. Med. Nauk SSSR 18:5:61-67. In Russian.

The site of synthesis of viral components and its chronological sequence were studied in a number of models. This work yielded a variety of data that apparently reflect a diversity in the structure and chemical composition of the viruses. These studies were carried out on models using myxoviruses and smallpox. Distribution of viral antigens was followed by FA and other methods.

III. TUMORVIRUS

A. ADENOVIRUS

5557

Huebner, R.J.; Chanock, R.M.; Rubin, B.A.; Casey, M.J. 1964. Induction by adenovirus Type 7 of tumors in hamsters having the antigenic characteristics of SV40 virus. *Proc. Nat. Acad. Sci.* 52:1333-1340.

As a portion of this broad study of virus-induced tumors, indirect fluorescent antibody was used to demonstrate nuclear fluorescence in cells from SV40-induced tumors. Serum from SV40 tumorous hamsters was used.

5558

Levinthal, J.D.; Ahmad-Zadeh, C.; van Hoosier, G.; Trentin, J. 1964. Immunofluorescence of human adeno-12 virus. *Proc. Amer. Ass. Cancer Res.* 5:40.

Fluorescein-labeled rabbit and anti-adeno-12 gamma globulin were used to follow the development and distribution of viral antigen in monolayers of normal cell cultures of human, simian, hamster, and murine origin. Hamster tumors induced in vivo with adeno-12 virus were similarly studied in vitro. In human cells (fetal fibroblast, lung, and kidney) antigen localized as two components in the nucleus. At the inner nuclear membrane antigen was demonstrable as bright dots and later homogeneous plaques that gradually coalesced to form a continuous broad irregular ring. In the center of the nucleus antigen as fine and coarse granules gradually became organized into a regular structure corresponding to the central inclusion body demonstrable in standard histologic staining.

5559

Levinthal, J.D.; Petersen, W. 1965. In vitro transformation and immunofluorescence with human adenovirus Type 12 in rat and rabbit kidney cells. *Federation Proc.* 24:260:174.

In vitro transformation of animal cells by a number of viruses has been reported and offers a system of carcinogenesis in which the early events of transformation may be observed. Kidneys of newborn Wistar rats and random-bred Picallilli rabbits were dispersed by trypsin, seeded in Leighton tubes containing cover slips, and grown to nearly confluent monolayers before infection with 310 to 325 tissue culture mean infective doses of human adenovirus Type 12, A-12, in arginine-enriched Eagle medium. After one month cells were maintained on a cloning-type medium. Cover slips were removed at 2, 10, and 14 days for studies using fluorescein-labeled rabbit anti-A-12 purified by DEAE cellulose chromatography. No

viral antigen was found. Transformation to a distinctive cell type and abnormal pattern of growth, resembling the appearance of hamster A-12 tumors grown in tissue culture, was observed in multiple foci after 70 days in rabbit cultures and 75 days in rat cultures. It is concluded that the capacity to undergo a complete viral cycle of replication is unnecessary for in vitro transformation, and that A-12 transformed cells closely resemble each other, whether of rat, rabbit, or hamster origin. Complete article.

5560

Melnick, J.L.; Rapp, F. 1965. Effect of inhibitors on induction of SV40 tumor antigen by an adenovirus-SV40 hybrid. Arch. Ges. Virusforsch. 1:424-435.

A Type 7 adenovirus-SV40 hybrid induces the synthesis of SV40 tumor or T antigen in hamster, rabbit, human, and monkey cells. Actinomycin D inhibits synthesis of the antigen suggesting that development of the antigen requires DNA-dependent RNA. Puromycin allows synthesis of T antigen, apparently a low molecular weight protein or polypeptide. Neither mitomycin C, fluorouracil, iododeoxyuridine, nor cytosin arabinoside prevents development of T antigen. It therefore appears probable that for synthesis of T antigen to proceed the synthesis of new virus DNA is not required regardless of whether this is virus DNA of SV40 or of adenovirus. Antiserum prepared against Type 7 adenovirus neutralizes the ability of the hybrid to induce SV40 tumor antigen. Antibody against the SV40 tumor antigen or against whole SV40 failed to do so. The evidence therefore suggests that the adenovirus hybrid is carrying a piece of SV40 tumor-inducing genome that replicates with the adenovirus. Adenovirus-SV40 hybrids have been found not only with Type 7 but also with many other types.

5561

O'Connor, G.T.; Rabson, A.S.; Malmgren, R.A.; Berezsky, I.K.; Paul, F.J. 1965. Morphologic observations of green monkey kidney cells after single and double infection with adenovirus 12 and simian virus 40. J. Nat. Cancer Inst. 34:679-693.

Sequential morphologic changes in African green monkey kidney (GMK) cells after single infection with adenovirus 12 and double infection with adenovirus 12 plus simian virus 40 (SV40) were studied by light and electron microscopy. A pronounced nuclear stippling was the first significant change noted in both groups 16 hours after infection. After 20 hours' incubation, adenovirus particles first appeared in those cultures infected with both viruses, and at 48 hours up to 80 per cent of the cells examined contained intranuclear adenovirus particles. This was in contrast to the group infected with adenovirus

12 only, in which virus particles were found in less than 5 per cent of cells even after 72 hours of incubation. The dissociation between viral synthesis and the nuclear manifestations of viral CPE was further illustrated by the absence of any specific viral antigen detectable by fluorescent antibody techniques in most cells of the singly infected group. Attempts to induce production of adenovirus particles in cells with altered nuclei, but without virus particles, by methods other than double infection with SV40 were not successful. Growth enhancement of adenovirus 12 by SV40 in GMK cells occurred only when the SV40 was added prior to, or simultaneously with, the adenovirus.

5562

Pope, J.H.; Rowe, W.P. 1964. Immunofluorescent studies of adenovirus 12 tumors and of cells transformed or infected by adenoviruses. *J. Exp. Med.* 120:577-588.

Complement-fixing antibody-positive sera from hamsters bearing tumors induced by adenovirus Type 12 (Ad. 12) revealed specific immunofluorescent-stainable antigens in essentially all Ad. 12 hamster tumor cells. The antigens were primarily in the form of cytoplasmic flecks; less frequent staining was seen as nuclear flecks or homogeneous staining of nucleus and cytoplasm of a small proportion of cells. Tumor cells did not stain with rabbit antisera to crude Ad. 12 virus or A and C antigens. The hamster serum also stained cytoplasmic flecks in an Ad. 12-induced BALB-c mouse tumor and Ad. 12-transformed hamster embryo tissue culture cells. The hamster serum also stained fleck-shaped antigens in hamster and human cell cultures inoculated with homologous and heterologous adenovirus types, although the hamster cells did not react with the rabbit Ad. 12 antiserum. Attempts to identify the fluorescent-stainable fleck-shaped antigens indicated that they are not previously recognized viral antigens and that the cytoplasmic antigens formed in hamster cell cultures inoculated with Ad. 12 are different from those in tumors and in acutely infected human cell cultures.

5563

Rapp, F.; Melnick, J.L.; Butel, J.S.; Kitahara, T. 1964. The incorporation of SV40 genetic material into adenovirus 7 as measured by intranuclear synthesis of SV40 tumor antigen. *Proc. Nat. Acad. Sci.* 52:1348-1352.

A strain of adenovirus 7 once contaminated with SV40 but now free of the infectious papovavirus was found to contain the genetic information necessary to induce synthesis of a new intranuclear antigen. The antigen, which can be detected by immunofluorescence and complement fixation, is immunologically identical to the tumor antigen induced by infectious SV40 in cells transformed by rabbit, and to hamster cells following

exposure of the cells to the virus; however, adenovirus penetration or replication seems required for synthesis of the new antigen. Information for synthesis of the antigen is transmitted to daughter cells during mitosis. One explanation of the observations recorded might be incorporation or hybridization of a portion of a virus genome, SV40, with the genome of an unrelated virus, adenovirus.

5564

Riggs, J.L.; Takemori, N.; Lennette, E.H. 1965. Detection of adenovirus Type 12 neoantigens in a continuous human amnion cell line, FL, by immunofluorescence. *Proc. Soc. Exp. Biol. Med.* 120:832-837.

Neoantigens produced in FL cells by infection with oncogenic types of adenoviruses were stained by the indirect FA technique with serum from hamsters bearing tumors induced by adenovirus Type 12 as the intermediate serum. The neoantigen of adenovirus Type 12 first appeared at 6 hours post-infection, and thereafter the amount within the cell, or nucleus, increased until 12 hours. The number of cells in which the antigen was demonstrable also increased until at 12 hours post-infection the majority of the cells contained the antigen. Formation of neoantigen preceded the formation of new infectious virus, as was shown by growth curve experiments and by staining with fluorescein-labeled antibody against the virus.

5565

Rowe, W.P.; Baum, S.G. 1964. Evidence for a possible genetic hybrid between adenovirus Type 7 and SV40 viruses. *Proc. Nat. Acad. Sci.* 52:1340-1347.

A passage line of Ad. 7 that had been freed of SV40 contamination induces an SV40 antigen in acutely infected tissue culture cells. The SV40 antigen-inducing capacity is integrally associated with the Ad. 7 infectious particle, suggesting incorporation of a portion of the SV40 genome into the Ad. 7 virion.

B. LEUKEMIA

5566

Brown, E.R. 1965. Fluorescent microscopy studies on a murine leukemia (S63) virus. *J. Lab. Clin. Med.* 66:20:859-860.

Previous work has shown that mice that recover from infection with the S63 virus develop specific antibodies that can be demonstrated by complement fixation, immunodiffusion, and passive cutaneous ana-

phylaxis tests. Normal mouse, convalescent mouse, normal rabbit, and anti-S63 rabbit sera were studied for comparative purposes. Gamma globulin was extracted from each of these sera by the cold-ethanol technique, further purified by Sober's method, and adsorbed against normal mouse or rabbit tissues prior to conjugation. The conjugate was adsorbed against silk fibroin to eliminate nonspecific fluorescence. The following normal and leukemic tissues of mice and humans were studied: spleen, liver, kidney, lymph node, and brain. The convalescent antibodies gave strongly fluorescent reactions with all the mouse leukemic tissues, less strong fluorescence with the human leukemic tissues, and no fluorescence with normal tissues. Fluorescence was limited to the cytoplasm of the cell, usually adjacent to the cell membrane. There was no nuclear fluorescence with these conjugates. The anti-S63 rabbit sera showed fluorescence with both normal and leukemic tissues even after adsorption by the normal tissues and normal serum. Virus particles were identified by electron microscopy within the cytoplasm and in the interstitial spaces of the same tissues, which showed fluorescence with specific conjugates. The antigen involved in the immunofluorescent reactions is probably the virus itself.

5567

Bryan, W.R.; Moloney, J.B.; O'Connor, T.E.; Fink, M.A.; Dalton, A.J. 1965. Viral etiology of leukemia. (Combined Clinical Staff Conference at the National Institutes of Health.) *Ann. Inter. Med.* 62:376-399.

Basic in vivo studies of leukemia immunity are reported. In addition, studies of the Rauscher virus using FA methods are revealing important aspects of the virus-leukemia relationship. It is believed that FA demonstrates an antigen of the interior of the virus particle. FA demonstrates cross-reaction between Rauscher and Moloney agents but not between Rauscher and Friend agents. It may be possible to serologically classify leukemic viruses.

5568

Fink, M.A.; Karon, M.; Rauscher, F.J.; Malmgren, R.A.; Orr, H.C. 1965. Further observations on the immunofluorescence of cells in human leukemia. *Cancer* 18:1317-1321.

FA prepared against 'virus-like particles' in human leukemic plasma reacted with 79 per cent of the bone marrow specimens from cases of acute leukemia in relapse but with only 25 per cent of the cases in remission. In six of eight cases followed through cycles of relapse-remission the fluorescent cells were not apparent in remission. FA prepared against the Rauscher murine leukemia virus showed positive reactions with the bone marrow cells from four cases of erythroleukemia and to some extent with bone marrow cells of a variety of other blood dyscrasias and malignancies. Three cases of human leukemia previously

have been shown to contain significant amounts of neutralizing antibody for the Rauscher murine leukemia virus, but no neutralizing antibody to this virus was detected in sera collected from several other human leukemia cases, contacts of cases or individuals who had been exposed to oncogenic viruses, including the Rauscher leukemia virus.

5569

Fink, M.A.; Malmgren, R.A. 1963. Fluorescent antibody studies of the viral antigen in a murine leukemia (Rauscher). J. Nat. Cancer Inst. 31:1111-1122.

Malignant lymphoid tissues of BALB/c mice and Osborne-Mendel rats infected with Rauscher virus (RNCI-2) contained viral antigen reactive with fluorescent antibody. The specific fluorescence occurred intranuclearly and less constantly in the cytoplasm. Absorption of the fluorescein-labeled rabbit anti-RNCI-2 globulin with infected mouse spleen, but no normal spleen, effectively prevented the specific fluorescence. In addition to the fluorescence in the malignant lymphoid cells, mature megakaryocytes of infected rat bone marrow contained specific nuclear, but no cytoplasmic, fluorescence. Cells from normal mice and rats, from transplantable lymphatic leukemias and reticulum cell sarcomas not known to be associated with a virus, and from DBA/2 mice infected with Friend virus did not react with the RNCI-2 antiserum.

5570

Fink, M.A.; Malmgren, R.A.; Rauscher, F.J.; Orr, H.C.; Karon, M. 1964. Application of immunofluorescence to the study of human leukemia. J. Nat. Cancer Inst. 33:581-588.

The concentrated plasma of individuals with leukemia has been shown by several electron microscopists to contain virus-like particles. In the present study, plasmas containing similar particles were used as an antigen in the production of an antiserum in rabbits. After absorption with normal human antigens and labeling with fluorescein isothiocyanate, this serum reacted specifically with various cellular elements of the bone marrow and peripheral blood of a significant number of patients with leukemia, and failed to react with leukocytes of nonleukemic individuals. A similar antibody prepared against a murine leukemia virus (Rauscher) cross-reacted with the bone marrow and peripheral blood cells from some humans with leukemia, and especially with the cells from a patient with erythroleukemia. It is of interest that this murine leukemia resembles human erythroleukemia pathologically. Tissue culture cells of Burkitt's lymphoma reacted strongly with the antihuman leukemia fluorescent antibody, and less strongly with the antimurine. These same preparations of fluorescent antibody did not react with HeLa cells infected with either herpes simplex or herpes zoster viruses. There may be antigenic similarities among strains of leukemia virus infecting various species. Viral etiology of human leukemia is hypothesized.

5571

Karon, M.R.; Fink, M.A.; Malmgren, R.A.; Rauscher, F.J.; Dalton, A.J.; Bryan, W.R.; Frei, E., III. 1964. Electron microscopic and immunofluorescent studies of viruses in human leukemia. *Blood* 24:835-836.

Approximately 25 per cent of patients with acute leukemia have virus-like particles in ultra-thin sections resembling those found in murine leukemia. Fluorescent antibodies prepared against plasma pellets from human and Rauscher leukemia in rodents have shown some correlations with the presence of clinical disease. Antigen from pooled human plasma pellets containing virus-like particles was used to prepare rabbit antisera. Globulin of this serum absorbed with normal human antigens was tagged with FITC. Anti-Rauscher virus serum was also prepared. Bone marrow smears from 72 patients with leukemia were tested. Forty-one reacted with both Rauscher and human antisera. Eight patients reacted with only antihuman and an additional eight patients with only anti-Rauscher. In positive reactors to antihuman serum the fluorescence correlated with abnormal cells in the marrow. Particularly strong fluorescence to anti-Rauscher serum was seen in three patients whose marrows had abnormal degrees of erythropoiesis. The serum of one of these patients neutralized 1.75 logs of Rauscher virus. No reactions were seen in the marrow cells of 24 of 25 normal controls or against tissue culture cells infected with herpes simplex, herpes zoster, Eaton agent, or influenza A. Antiserum made against a pool of plasma pellets from normal subjects failed to react with bone marrow cells known to be positive. These findings support the hypothesis of a viral etiology for human leukemia.

5572

Old, L.J.; Boyse, E.A. 1965. Antigens of tumors and leukemias induced by viruses. *Federation Proc.* 24:1009-1017.

Various types of viruses are considered in their relationship to cancer. Among these are SV40, L.L. adenovirus Type 7, Rous sarcoma virus, mammary tumor virus, mouse leukemia, and Friend virus. Various techniques are discussed that demonstrate specific tumor and leukemia antigens. FA is a prime method. Nuclear FA staining is common in leukemia cells. Origins of specific antigens are discussed. The significance of antigens found in tumors or leukemias should not be discounted on grounds that these same antigens may be found in apparently normal tissues.

5573

Pasternak, G. 1965. Serologic studies on cells of Graffi virus-induced myeloid leukemia in mice. *J. Nat. Cancer Inst.* 34:71-84.

Sera of mice resistant to isologous inocula of Graffi virus-induced myeloid leukemias reacted in vitro with cells of leukemias having the same viral origin. Specific humoral antibodies were detected by the indirect fluorescent antibody and cytotoxic tests. Antibody activity was removed by absorption of the immune sera with Graffi leukemia cells. Normal spleen and lymph node cells and cells of a spontaneous AKR leukemia did not absorb the activity directed against Graffi leukemia cells. The immune sera possessed virus-neutralizing capacity as indicated by the inactivation of a virus-containing, cell-free leukemia filtrate. Formation of antibodies reacting with leukemia cells was also induced by inoculation of adult mice with both active and formalin-treated preparations of the Graffi virus. Whether the specific antigen of the leukemia cells could represent an antigen present in the virus is discussed.

5574

Rapp, F.; Friend, C. 1963. Early detection and localization of Swiss mouse leukemia virus. *Acta Unio Int. Contra Cancr.* 19:348-350.

The growth cycle of a virus causing leukemia in adult Swiss mice has been studied with immune serum and with the indirect immunofluorescent technique. Virus antigens were first observed in the nucleus and perinuclear area of single or small groups of leukemic cells. Progress of the infection resulted in a shift of antigen to the cytoplasm and ever larger foci of cells were found to be involved. Detection of infectious virus by mouse titrations correlated with the detection of virus antigens in the liver of infected mice. These results confirm and extend initial findings made with the electron microscope.

5575

Tyndall, R.L.; Vidrine, J.G.; Teeter, E.; Upton, A.C.; Harris, W.W.; Fink, M.A. 1965. Cytopathogenic effects in a cell culture infected with a murine leukemia virus. *Proc. Soc. Exp. Biol. Med.* 119:186-189.

Cell cultures grown in modified NCTC 109 medium and infected with the Rauscher leukemia virus showed cytopathogenic effects when subcultured in Eagle basal medium. Noninfected control cultures treated in a similar manner showed no such effect. The cytopathogenic effects in infected cultures included increased cytoplasmic eosinophilia in the form of either eosinophilic granules or inclusion bodies, cytoplasmic vacuolization, and marked variation in the size, shape, and intensity of staining of cell nuclei. FA demonstrated staining cytoplasmic and nuclear inclusions.

C. PAPOVAVIRUS SV40

5576

Ashkenazi, A.; Melnick, J.L. 1963. Tumorigenicity of simian papovavirus SV40 and of virus-transformed cells. *J. Nat. Cancer Inst.* 30:1227-1265.

The tumorigenicity of the simian papovavirus SV40 and of virus-transformed cells was investigated in hamsters. A genetically purified stock of the virus readily produced tumors in newborn hamsters, and the virus could be recovered from most tumors. About half the animals developed antibodies against the virus. Those failing to make antibodies did not become tolerant to a later challenge of the virus. Virus-free tumors were transplanted serially in hamsters, but no virus could be recovered from the transplanted tumors. The recipient animals did not produce SV40 antibody when tested 2 to 3 months after the tumors appeared. SV40 virus transformed human fibroblast cultures derived from embryonic lung. Cells changed from fibroblastic to epithelioid. SV40 was produced by the infected lung fibroblasts, but the virus yield declined when the cells became epithelioid. Transformation in vitro of hamster embryo cells was produced by SV40. The transformed cells produced tumors when inoculated in hamsters by various routes. No virus was recovered from these cells or from the tumors produced by them. Virus-induced primary neoplasms were less cellular than the ones produced by the hamster embryo cells transformed in vitro. The cells from these tumors when grown in tissue culture and the transformed cells maintained for long periods in culture had the same appearance. Fluorescent antibody examination of sections from the tumor yielding the highest concentration of virus revealed isolated cells having brilliant yellow-green fluorescence. The fluorescence was concentrated in the nuclei of the cells. Others have found the fluorescence in the perinuclear zone and cytoplasm. This may be due to observation at different stages in the growth cycle of the virus. In the beginning of the infection viral antigen is in the nucleus, and at the end stages diffuse antigen is throughout the cytoplasm.

5577

Beardmore, W.B.; Havlich, M.J.; Serafine, A.; McLean, I.W., Jr. 1965. Interrelationship of adenovirus Type 4 and papovavirus SV40 in monkey kidney cell cultures. *J. Immunol.* 95:422-435.

Our experimental data indicate pure Type 4 adenovirus will not produce infectious progeny in monkey kidney cell culture; it will reproduce in HEK and Hep-2 cells and give high yields of infectious virus. The incomplete form of adenovirus developed in monkey kidney tissue is associated with a lack of formation of intranuclear RNA; a dual infection of Type 4 adenovirus and SV40 in primary monkey kidney cell culture allows the adenovirus to mature in an apparently normal manner and simultaneous massive infection of monkey kidney cell cultures with SV40 is required for maximum growth of Type 4 adenovirus. Indirect fluorescent antibody was employed to detect viral antigen in the cells.

5578

Black, P.H.; Rowe, W.P. 1965. Induction of SV40 T antigen with SV40 DNA. *Virology* 27:436-439.

SV40 T antigen can be induced by infection of cells with SV40 DNA. Therefore, it is probable that the genetic information that codes the T antigen is present in the viral genome. SV40 T antigen was detected in tissue culture cells by indirect FA. Direct FA was used to detect SV40 viral antigen in cells. Infected nuclei were counted with the FA marker. The induction of T antigen was visually followed.

5579

Butel, J.S.; Rapp, F. 1965. The effect of arabinofuranosylcytosine on the growth cycle of simian virus 40. *Virology* 27:490-495.

Arabinofuranosylcytosine, at concentrations of 1 and 10 ug per ml, inhibits the synthesis in green monkey kidney cells of SV40 viral antigen and infectious virus but permits the production of SV40 tumor antigen. The tumor antigen can be detected by 18 hours postinfection but addition of the inhibitor as late as 21 hours postinfection prevents virus synthesis. Yields of infectious virus are depressed even when arabinofuranosylcytosine is added 24 or 30 hours after infection. Inhibition can be reversed by removal of the compound. After removal of the inhibitor, the time required for synthesis of virus was similar to that observed between synthesis of tumor antigen and virus antigen in non-inhibited cultures. The concomitant addition of deoxycytidine triphosphate and arabinofuranosylcytosine to inhibited cultures also reversed the inhibition, a finding compatible with the working hypothesis that arabinofuranosylcytosine can inhibit a step in the synthesis of virus DNA. It therefore appears likely that synthesis of SV40 tumor antigen does not require replication of virus DNA. Direct FA was used to stain viral antigen, and indirect FA was employed to stain tumor antigen.

5580

Chumakov, M.P.; Mustafina, A.N.; Chumakova, M.Y.; Karmysheva, V.Y.; Shestopalova, N.I.; Reingold, V.N. 1964. Cultivation of simian virus SV40 in continuous human diploid cells. *Acta Virol.* 8:217-224.

Investigations were carried out on persistent infection of human diploid cell strains with vacuolating simian virus (SV40) causing cell transformation. Transformation occurred only in cultures inoculated with undiluted virus suspensions or with suspensions diluted 1:10. Inoculation with suspensions diluted 1:1000 or more did not cause transformation within 15 weeks. Inoculation of suspensions of the same

cells resulted in acute infection with the formation of symplasts. Electron microscopic investigations of ultra-thin sections of human diploid cells from cultures inoculated with undiluted virus suspension revealed scattered aggregates of SV40 particles. No such particles were detected at the same intervals in cultures of the same cells inoculated with smaller doses of virus. FA was employed to detect viral antigen.

5581

Diderholm, H. 1963. A fluorescent antibody study on the formation of simian virus 40 in monkey kidney cells. *Acta Pathol. Microbiol. Scand.* 57:348-352.

The indirect fluorescent antibody technique was used to study the formation of simian virus 40 (SV40) in kidney cell cultures of the African green monkey. A few nuclei contained specific fluorescent material 2 days after infection. During the next 3 days more nuclei were affected and the intensity of the fluorescence increased. Thereafter a specific cytoplasmic fluorescence appeared that increased with time; the nuclear fluorescence decreased or disappeared.

5582

Dzagurov, S.G.; Chumakov, M.P.; Karmysheva, V.Ya.; Kolyaskina, G.I.; Kurochkina, N.P.; Panosyan, L.B. 1963. The use of the fluorescent antibody method to locate SV40 in the cell and to reveal it quickly in vaccine. *Vop. Virusol.* 8:471-474. In Russian.

By the direct fluorescent antibody method even minimum amounts of SV40 can be revealed during the first 120 hours after infection in cultures of trypsinized kidneys of the grivet monkey diluted up to 10⁻¹². By this method two stages of the accumulation of SV40 in an affected cell are detected - the nuclear and cytoplasmic ones. To detect SV40 in live poliomyelitis vaccine, prepared by using Macaca rhesus monkey tissue cultures, two methods were studied and compared: the cytopathic effect and the fluorescent antibody method. Positive results of the detection of SV40 obtained by the cytopathic effect coincided 100 per cent with those revealed by the fluorescent antibody method, but of 26 harvests of vaccines that were negative by the cytopathic effect, 10 were positive by the fluorescent antibody method. The total number of harvests of vaccines positive by the fluorescent antibody method was 64.4 per cent, as against 42.2 per cent by the cytopathic effect. These data show that the first method is evidently more sensitive for revealing SV40; however, further studies are necessary to confirm its specificity.

5583

Feldman, L.A.; Melnick, J.L.; Rapp, F. 1965. Influence of SV40 genome on the replication of an adenovirus-SV40 hybrid population. *J. Bacteriol.* 90:778-782.

Replication of a Type 7 adenovirus-SV40 hybrid population in primary African green monkey kidney cells was accompanied by the formation of SV40 tumor antigen, adenovirus antigens, and cytopathic changes characteristic of adenovirus infection. Prior infection of the cultures with SV40 stimulated replication of nonintegrated adenovirus Type 7 but did not enhance the replication of the hybrid virus. These results suggest that the population of the adenovirus-SV40 hybrid studied contains many particles carrying SV40 information. Replication of SV40 virus was not enhanced by co-infection with nonintegrated adenovirus Type 7 or with the hybrid. Cytosine arabinoside strongly inhibited replication of the hybrid population in African green monkey kidney cells. Enhanced replication of nonintegrated adenovirus Type 7 by SV40 was blocked by cytosine arabinoside; this block could be reversed by 2-deoxycytidine or deoxycytidine triphosphate.

5584

Fernandes, M.V.; Moorhead, P.S. 1965. Transformation of African green monkey kidney cultures infected with simian vacuolating virus (SV40). *Texas Rep. Biol. Med.* 23:242-258.

Cultures derived from African green monkey kidney cells were infected with SV40. A typical SV40 cytopathic effect was observed when using as inoculum a high multiplicity of virus; only intranuclear inclusions could be detected with a very low multiplicity of virus. Cultures infected in the latter manner could be subcultivated and maintained in a carrier state. These infected cultures showed signs of transformation 15 weeks after exposure of the virus. Colonies of morphologically abnormal cells with an irregular pattern and loss of mitotic inhibition increased progressively in these cultures. Gross chromosomal abnormalities and the evolution of a subdiploid population accompanied the transformation of the infected line. Uninfected controls did not undergo more than minor departures from the original diploid condition until after its first year of in vitro cultivation. Virus could not be detected after the 36th subculture of the transformed cultures. Detection of the specific complement-fixing antigen indicates that the transformation of infected cells was caused by the inoculation of SV40.

5585

Gilden, R.V.; Carp, R.I.; Taguchi, F.; Defendi, V. 1965. The nature and localization of the SV40-induced complement fixing antigen. *Proc. Nat. Acad. Sci.* 53:684-691.

Indirect FA was used to locate virus antigen in the nuclei of transformed human and hamster cells. The life cycle of the virus was also followed by FA. Many other properties of the virus are described.

5586

Huebner, R.J.; Chanock, R.M.; Rubin, B.A.; Casey, M.J. 1964. Induction by adenovirus Type 7 of tumors in hamsters having the antigenic characteristics of SV40 virus. *Proc. Nat. Acad. Sci.* 52:1333-1340.

As a portion of this broad study of virus-induced tumors, indirect fluorescent antibody was used to demonstrate nuclear fluorescence in cells from SV40-induced tumors. Serum from SV40 tumorous hamsters was used.

5587

Ito, M.; Ikegami, M.; Shiroki, K.; Tagaya, I. 1964. Studies on the multiplication of simian virus 40 (vacuolating virus) by means of fluorescent antibody technique. *Jap. J. Med. Sci. Biol.* 17:179-193.

Multiplication of SV40 in African green monkey kidney cells was investigated by FA. In both primary and continuously cultured green monkey kidney cells, the specific antigen of SV40 was first detected in the nucleus 1 to 2 days after infection. The intranuclear antigen was observed as fluorescent granules of various sizes and its concentration reached the maximum 2 to 4 days after infection. At that time the viral antigen could also be observed in the cytoplasm and the cytoplasmic vacuolization became observable in a small number of cells. This suggested that SV40 was mainly synthesized in the nucleus and then transferred into the cytoplasm, giving rise to the vacuolization in the cytoplasm. The growth of SV40 in continuously cultured green monkey kidney cells was investigated in regard to the cell-associated virus and the fluid virus. The course of the appearance and increase of the specific viral antigen detectable by the fluorescent antibody technique paralleled the growth curves of infectious virus. Human continuously cultured cell lines FL, Hep-2, and HeLa-S3 supported the growth of SV40. The ratio of the fluorescent cells to the total cells in the culture was usually proportionate to the infectivity of the culture fluid.

5588

Ito, M.; Ikegami, M.; Shiroki, K.; Tagaya, I. 1964. Variable susceptibility of cynomolgus monkey kidney cells to the specific cytopathic effect of simian virus 40. *Jap. J. Med. Sci. Biol.* 17:283-294.

The specific vacuolation was found in the cytoplasm of some batches of cynomolgus monkey kidney cells following SV40 infection, which was indistinguishable from that observed in African green monkey kidney cells. But the other batches of the cells examined did not show any such cytopathic effect. Both types of the cynomolgus monkey kidney cell cultures supported equally a full growth of SV40. The susceptibility to SV40-vacuolation was supposed to be one of the special cellular characters that might be genetically controlled. Viral antigen was demonstrated in cells by FA.

5589

Khera, K.S.; Ashkenazi, A.; Rapp, F.; Melnick, J.L. 1963. Immunity in hamsters to cells transformed in vitro and in vivo by SV40: Tests for antigenic relationship among the papovaviruses. *J. Immunol.* 91:604-613.

The inoculation of SV40-transformed cells into hamsters previously inoculated with one of the papovaviruses indicated a high degree of protection conferred by the homologous SV40 virus and none by polyoma virus. The human wart virus and the Shope papilloma virus seemed to produce a limited degree of protection. Hamster sera were used in indirect FA tests to detect antibodies to SV40 virus and reaction against H-50 cells. SV40 was not very antigenically potent. An anti - H-50 cell reaction was observed but could not be explained.

5590

Kitahara, T.; Butel, J.S.; Rapp, F.; Melnick, J.L. 1965. Correlation between complement-fixing cell antibody and immunofluorescent nuclear antibody in hamsters bearing SV40-induced tumors. *Nature* 205:717.

FA and CF tests were used to titer hamster antibody production against SV40 tumor cell antigen. A high degree of correlation was seen between antibodies detected by the two methods. Both antibodies must develop simultaneously in hamsters carrying the SV40 tumors or the two different methods are detecting the same antibody. If this is true, then the complement-fixing antibody would be the result of the immune response of the animals to the particulate, intranuclear, viral-induced antigen present in all virus-free transformed cells. If the antigens detectable by complement fixation and immunofluorescence elicit the same antibody, then the intracellular site of synthesis of the complement-fixing antigen would also be the nucleus, and the antigen would be produced in every cell of a transformed culture.

5591

Kitahara, T.; Melnick, J.L.; Rapp, F. 1965. Relationship between specific complement-fixing and intranuclear antigens in SV40-induced tumors. *Bacteriol. Proc.* V66:108.

The relationship of this CF antigen to the new intranuclear antigen found by immunofluorescence techniques in cells transformed by this virus was investigated by testing the sera from numerous hamsters by both complement fixation and immunofluorescence against extracts and cells from hamster tumors. Sera found to be positive by immunofluorescence were also almost always positive by complement fixation; sera negative in one system were also negative in the second. Animals with tumors less than 8 mm in diameter rarely developed detectable antibody. As the tumors developed to a larger size, a high percentage of the sera became positive, but a number of animals with large tumors failed to develop antibody. The cells from the tumors of these antibody-negative animals contained both the complement-fixing and intranuclear antigen. Hamsters can be vaccinated with live SV40 so that they usually become resistant to SV40-transformed cells. A significant number of vaccinated hamsters that developed tumors after cell challenge failed to produce antibody against the SV40 tumor antigen as measured by complement fixation and immunofluorescence. Results strongly suggest that the complement-fixing cellular antigen and intranuclear antigen detected by immunofluorescence are immunologically similar or identical.

5592

Levine, S.I.; Goulet, N.R.; Liu, O.C. 1965. Beta-propiolactone decontamination of simian virus 40 as determined by a rapid fluorescent antibody assay. *Appl. Microbiol.* 13:70-72.

Beta-propiolactone vapor treatment of vaccine production facilities has been shown to be approximately 90 per cent effective in the elimination of large quantities of simian virus 40 (SV40). The use of a rapid fluorescent antibody assay for the detection of SV40 was also studied.

5593

Levinthal, J.D.; Shein, H.M. 1963. Morphology and distribution of SV40-infected cells in human, simian and hamster renal cell cultures as detected by immunofluorescence. *Proc. Soc. Exp. Biol. Med.* 112:405-409.

Viral antigen is found in the nucleus in kidney tissue cultures of SV40-infected grivet monkey, rhesus monkey, primary fetal human, SV40-transformed fetal human, and primary newborn Syrian hamster. Because of aggregation of antigen around what appears to be altered nucleoli in all four tissues it is suggested that viral synthesis and assembly may occur in the nucleolus. In the four tissues studied there is an inverse correlation between the proportion of cells exhibiting specific nuclear fluorescence and the

brilliance of fluorescence and ease of induction of transformation in vitro. No specific nuclear fluorescence was observed in SV40-transformed Syrian hamster renal cultures even when superinfected with SV40 virus.

5594

Melnick, J.L.; Rapp, F. 1965. Effect of inhibitors on induction of SV40 tumor antigen by an adenovirus-SV40 hybrid. Arch. Ges. Virusforsch. 17:424-435.

A Type 7 adenovirus-SV40 hybrid induces the synthesis of SV40 tumor or T antigen in hamster, rabbit, human, and monkey cells. Actinomycin D inhibits synthesis of the antigen suggesting that development of the antigen requires DNA-dependent RNA. Puromycin allows synthesis of T antigen, apparently a low molecular weight protein or polypeptide. Neither mitomycin C, fluorouracil, iododeoxyuridine, nor cytosine arabinoside prevents development of T antigen. It therefore appears probable that for synthesis of T antigen to proceed the synthesis of new virus DNA is not required regardless of whether this is virus DNA of SV40 or of adenovirus. Antiserum prepared against Type 7 adenovirus neutralizes the ability of the hybrid to induce SV40 tumor antigen. Antibody against the SV40 tumor antigen or against whole SV40 failed to do so. The evidence therefore suggests that the adenovirus hybrid is carrying a piece of SV40 tumor-inducing genome that replicates with the adenovirus. Adenovirus-SV40 hybrids have been found not only with Type 7 but also with many other types.

5595

Melnick, J.L.; Stinebaugh, S.E.; Rapp, F. 1964. Incomplete simian papovavirus SV40: Formation of noninfectious viral antigen in the presence of fluorouracil. J. Exp. Med. 119:313-326.

A study was made of the effects of 5-fluorouracil (FU) and 5-fluorodeoxyuridine (FUDR) on the replication of the simian papovavirus SV40 in Cercopithecus monkey kidney cells and on the production of virus antigen by these cells. Both drugs suppressed production of new infectious virus by SV40-infected cells. Synthesis of viral protein was also suppressed by FUDR, but not by FU. In the presence of FU, infected cells produced large amounts of viral protein that were detected by FA. The antigen was not distributed in a particulate fashion as in untreated cells. Diffuse virus antigen was observed in the nuclei of FU-treated cells, resembling the distribution of antigen near the end of the eclipse period in untreated, infected cultures. This stage of antigen production presumably preceded viral assembly. Virus particles were rarely seen with the electron microscope in infected FU-treated cells, although large numbers of SV40 particles were readily visualized in untreated, infected cells. It appears that at least one antigenic protein of this papovavirus is synthesized abundantly in FU-treated cells, but is not assembled into virus shells in the presence of the inhibitor.

5596

Moore, R.D.; Schoenberg, M.D. 1964. The response of the histiocytes and macrophages in the lungs of rabbits injected with Freund's adjuvant. *Brit. J. Exp. Pathol.* 45:488-497.

The cellular response in the lungs of rabbits given complete Freund's adjuvant intravenously was considered in relation to the appearance and kind of circulating antibody, the presence of antigen and antibody in the cells, and the development of tissue sensitivity to the mycobacteria. The proliferation of histiocytes in the alveolar walls, the accumulation of macrophages in the alveolar spaces, the appearance of polymorphonuclear leukocytes, lymphocytes and plasma cells, and the formation of granulomata in the lungs occurred before any specific immunological reaction was recognized. The large mononuclear cells of the granulomata differed from the macrophages and histiocytes both structurally and functionally. Circulating antibody and skin sensitivity were not detected until the cellular reaction was well established. The presence of plasma cells containing gamma globulin at a time when 7S antibody appears in the circulation is described.

5597

O'Connor, G.T.; Rabson, A.S.; Malmgren, R.A.; Berezesky, I.K.; Paul, F.J. 1965. Morphologic observations of green monkey kidney cells after single and double infection with adenovirus 12 and simian virus 40. *J. Nat. Cancer Inst.* 34:679-693.

Sequential morphologic changes in African green monkey kidney (GMK) cells after single infection with adenovirus 12 and double infection with adenovirus 12 plus simian virus 40 (SV40) were studied by light and electron microscopy. A pronounced nuclear stippling was the first significant change noted in both groups 16 hours after infection. After 20 hours' incubation, adenovirus particles first appeared in those cultures infected with both viruses, and at 48 hours up to 80 per cent of the cells examined contained intranuclear adenovirus particles. This was in contrast to the group infected with adenovirus 12 only, in which virus particles were found in less than 5 per cent of cells even after 72 hours of incubation. The dissociation between viral synthesis and the nuclear manifestations of viral CPE was further illustrated by the absence of any specific viral antigen detectable by fluorescent antibody techniques in most cells of the singly infected group. Attempts to induce production of adenovirus particles in cells with altered nuclei, but without virus particles, by methods other than double infection with SV40 were not successful. Growth enhancement of adenovirus 12 by SV40 in GMK cells occurred only when the SV40 was added prior to, or simultaneously with, the adenovirus.

5598

Pope, J.H.; Rowe, W.P. 1964. Detection of specific antigen in SV40-transformed cells by immunofluorescence. *J. Exp. Med.* 120:121-128.

With an immunofluorescent technique involving the use of serum of hamsters with SV40 tumors, nuclear fluorescence was detected in each of five cell lines, derived from four mammalian species, transformed by SV40 virus. Essentially all nuclei, including those of multinuclear cells, were fluorescent-stainable. Serum of hamsters bearing SV40 tumors also gave nuclear fluorescence in susceptible cells, AGMK or BSC-1, acutely infected with SV40 virus. These findings provide further evidence that cellular incorporation of the SV40 viral genome, with partial expression of the genome by synthesis of at least one virus-specific antigen, is an integral property of all SV40-transformed cells.

5599

Rapp, F.; Butel, J.S.; Feldman, L.A.; Kitahara, T.; Melnick, J.L. 1965. Differential effects of inhibitors on the steps leading to the formation of SV40 tumor and virus antigens. *J. Exp. Med.* 121:935-944.

The effect of DNA antagonists and various antibiotics on steps in the synthesis of SV40 virus in green monkey kidney cells was investigated. Both the early-forming tumor (T) antigen and the later-synthesized virus (V) antigen were synthesized in the presence of fluorouracil and iododeoxyuridine. Cytosine arabinoside, and fluorodeoxyuridine in starved cells, prevented synthesis of V antigen but not of T antigen. The synthesis of T antigen, therefore, does not require synthesis of virus DNA. Virus particles formed only in the presence of the iododeoxyuridine and they were noninfectious. Actinomycin D inhibited synthesis of both tumor and virus antigens, suggesting that the synthesis of these antigens involved DNA-dependent RNA. Puromycin allowed synthesis of the T antigen, which remained localized at the nucleolar membrane. This finding with puromycin suggests that the T antigen is a protein of low molecular weight. Virus antigen forming in the presence of mitomycin C, *p*-fluorophenylalanine, iododeoxyuridine, or fluorouracil was distributed atypically. These inhibitors caused the V antigen to be diffusely spread throughout the nucleus or to be concentrated at the nuclear membrane.

5600

Rapp, F.; Butel, J.S.; Melnick, J.L. 1964. Virus-induced intranuclear antigen in cells transformed by papovavirus SV40. *Proc. Soc. Exp. Biol. Med.* 116:1131-1135.

Hamsters bearing virus-free tumors induced by cells transformed in vitro or in vivo by papovavirus SV40 produce circulating antibody capable of reacting with intranuclear antigens synthesized by SV40-

transformed cells. The reaction is particulate and does not involve the nucleolus. All cells in transformed cultures, regardless of whether they are of hamster or of human origin, synthesize the antigen. Indirect FA was used to demonstrate the intracellular viral antigen.

5601

Rapp, F.; Melnick, J.L.; Butel, J.S.; Kitahara, T. 1964. The incorporation of SV40 genetic material into adenovirus 7 as measured by intranuclear synthesis of SV40 tumor antigen. *Proc. Nat. Acad. Sci.* 52:1348-1352.

A strain of adenovirus 7 once contaminated with SV40 but now free of the infectious papovavirus was found to contain the genetic information necessary to induce synthesis of a new intranuclear antigen. The antigen, which can be detected by immunofluorescence and complement fixation, is immunologically identical to the tumor antigen induced by infectious SV40 in cells transformed by rabbit, and to hamster cells following exposure of the cells to the virus; however, adenovirus penetration or replication seems required for synthesis of the new antigen. Information for synthesis of the antigen is transmitted to daughter cells during mitosis. One explanation of the observations recorded might be incorporation or hybridization of a portion of a virus genome, SV40, with the genome of an unrelated virus, adenovirus.

5602

Rapp, F.; Melnick, J.L.; Kitahara, T. 1965. Tumor and virus antigens of simian virus 40: Differential inhibition of synthesis by cytosine arabinoside. *Science* 147:625-627.

Cells infected with papovavirus SV40 not only synthesize viral antigen but also synthesize the specific nonviral antigen found in SV40-induced tumors. In the presence of the DNA antagonist cytosine arabinoside, infected cells fail to make viral antigen but still synthesize the tumor antigen. Iododeoxyuridine does not inhibit the synthesis either of tumor or of virus antigen but does prevent the development of infectious virus.

5603

Rapp, F.; Melnick, J.L.; Kitahara, T.; Sheppard, R. 1965. Search for virus-induced antigens in human tumors using the SV40-hamster system as a model. *Proc. Soc. Exp. Biol. Med.* 118:573-576.

Cell cultures derived from various types of human cancers were examined for the presence of tumor-specific antigens by both immunofluorescence and complement fixation methods, in which the serum of the patient was tested against cells grown from his cancer. The methods, previously used successfully to detect a new intranuclear antigen induced by the papovavirus SV40 in transformed human and hamster cells and in hamster tumors failed to reveal the presence of immunologically reactive components.

5604

Rowe, W.P.; Baum, S.G. 1964. Evidence for a possible genetic hybrid between adenovirus Type 7 and SV40 viruses. *Proc. Nat. Acad. Sci.* 52:1340-1347.

A passage line of Ad. 7 that had been freed of SV40 contamination induces an SV40 antigen in acutely infected tissue culture cells. The SV40 antigen-inducing capacity is integrally associated with the Ad. 7 infectious particle, suggesting incorporation of a portion of the SV40 genome into the Ad. 7 virion.

5605

Rowe, W.P.; Baum, S.G. 1965. Studies of adenovirus SV40 hybrid viruses: II. Defectiveness of the hybrid particles. *J. Exp. Med.* 122:955-966.

E46 virus purified by limiting dilution and plaque isolation of African green monkey kidney (AGMK) cells consists of a mixture of hybrid and non-hybrid virus particles. Of 102 plaque isolates obtained in HEK cells, 98 contained no detectable hybrid virus and four contained a small proportion of hybrid virus. Plaque isolates from AGMK cells uniformly induced SV40 neoantigen, but when these plaque isolates were plaqued in human embryonic kidney (HEK) cells the progeny were again non-hybrid adenovirus. It thus appears impossible to obtain a pure line of hybrid virus. The proportion of hybrid virus produced in HEK cells fell progressively with decreasing multiplicity of infection, and plaque induction by E46 virus in AGMK followed two-hit kinetics, indicating that the hybrid virus particles are defective and require dual infection with non-hybrid virus for their propagation.

5606

Rowe, W.P.; Baum, S.G.; Pugh, W.E.; Hoggan, M.D. 1965. Studies of adenovirus SV40 hybrid viruses. *J. Exp. Med.* 122:943-954.

The titers of adenovirus and SV40 genetic carriers in hybrid preparations, E46, can be quantitated by determining the percentage of cells showing neoantigens stainable with fluorescent antibody at 21 to 24 hours; both titers can be obtained with a single cover slip. The adenovirus and SV40 antigen-inducing titers so obtained are of the same order of magnitude in stock preparations of E46 grown in either African green monkey kidney (AGMK) or human embryonic kidney tissue culture (HEK) and are generally within one log of the infectivity titer. Quantitative studies of 50 C heat inactivation, ultracentrifugation, and equilibrium density gradient centrifugation of E46 gave no indication that SV40 neoantigen induction could be dissociated from adenovirus, but adenovirus and SV40 virus grown as a mixed infection were

readily dissociated by these procedures. Pretreatment of HEK cells with a medium containing 5-fluorouracil deoxyriboside did not affect induction of either adenovirus or SV40 neoantigen or development of cytopathic effects after infection with E46, but did prevent formation of adenovirus 7 viral antigen.

5607

Tevethia, S.S.; Katz, M.; Rapp, F. 1965. New surface antigen in cells transformed by simian papovavirus SV40. *Proc. Soc. Exp. Biol. Med.* 119:896-901.

Hamster cells transformed by papovavirus SV40 in vitro and in vivo possess new surface antigens that can be detected by the indirect immunofluorescent technique. Antibody against the surface antigens of the SV40-transformed cells fails to react with normal cells from various species including the hamster, with the spontaneously oncogenic hamster BHK21 cells, or with hamster cells transformed by adenovirus Type 12. Inoculation of hamsters with cells not transformed by SV40 did not elicit synthesis of an antibody capable of reacting with the surface antigens of the cells transformed by the papovavirus.

5608

Tevethia, S.S.; Rapp, F. 1965. Detection of an antibody reacting with surface antigens of cells transformed by papovavirus SV40. *Bacteriol. Proc.* V67:108.

Antibodies capable of reacting with intranuclear antigens in SV40-transformed cells have been demonstrated in the sera of hamsters bearing tumors induced by cells transformed by the virus. Since such antibodies do not enter viable cells and, furthermore, are not found in hamsters that fail to develop tumors, studies were carried out on the protective mechanism in hamsters vaccinated with live SV40. Vaccinated animals resistant to transplantation were rechallenged with various hamster cells, and sera were examined for ability to react with cells by immunofluorescence methods. Hamsters previously immunized with SV40 responded specifically to this second challenge with SV40-transformed cells by forming antibody against cell surface antigens. This antibody did not react with normal hamster cells nor with the oncogenic BHK21 hamster cells. Challenge of the vaccinated, resistant hamsters with the latter cells did not elicit antibody against surface antigens of the SV40-transformed cells. Sera of unvaccinated hamsters developing tumors after inoculation of transformed cells had no detectable antibody against cell surface antigens, even though they were positive for antibodies against the intranuclear tumor antigen. This investigation has demonstrated the development of antibodies directed specifically against surface antigens of SV40-transformed cells. These antibodies may play a role in the rejection of the tumor cells in vivo.

D. POLYOMA

5609

Adzhigitov, F.I. 1964. Some types of polyoma virus interaction with cells of various tissue cultures. Vop. Virusol. 9:667-670. In Russian.

Polyoma virus behavior was studied in 18 types of tissue cultures: embryonal tissues of hen, mouse, swine, cow, macaca rhesus, and man; primary kidney tissue cultures of chicks, mice, and monkeys, established cell lines KEM-1, APO, SOTs, and HeLa. Three types of virus cell interaction were observed: multiplication of virus accompanied by cytopathic effect, multiplication of virus on the pattern of latent infection, and absence of virus multiplication. The character of the interaction of polyoma virus with the above-mentioned cultures is discussed, considering the influence of such factors as specific origin of cultures, genetics, age, and other factors.

5610

Bereczky, E.; Hughes, R.; Bowen, J.M.; Munyon, W.; Dmochowski, L. 1965. Study of DNA synthesis and antigen formation in polyoma virus - infected mouse embryo cells by autoradiography and immunofluorescence. Texas Rep. Biol. Med. 23:3-15.

Autoradiography and immunofluorescence techniques have been used to study the synthesis of DNA and the formation of viral protein in polyoma virus - infected mouse embryo cells. When the cells were pretreated and maintained on a low-serum-containing medium, DNA synthesis in non-infected cultures was kept at a low level. DNA synthesis in polyoma virus - infected cultures maintained on a similar medium began about 18 hours after infection and remained at a higher level than that of control cultures for approximately 15 hours. The DNA synthesis in the infected cells preceded the appearance of mature virus particles. The number of cells showing DNA synthesis as measured by tritium-labeled nuclei exceeded the number of cells showing formation of virus antigen, as measured by fluorescent antibody staining.

5611

Diamond, Leila; Crawford, L.V. 1964. Some characteristics of large-plaque and small-plaque lines of polyoma virus. Virology 22:235-244.

Suspensions of small-plaque and large-plaque lines of polyoma virus showed marked differences in the adsorption of virus particles to cells and to the hemagglutinin inhibitor released from cells. Small-plaque virus was more readily adsorbed than large-plaque virus, and this difference could be used as an adjunct to plaque size distribution in

determining the predominant plaque type of virus suspensions. The suspensions of large- and small-plaque virus contained similar proportions of full particles of buoyant density 1.32 and empty particles of buoyant density 1.29. Indirect FA staining was used to demonstrate polyoma virus in hamster kidney cells.

5612

Fraser, K.B.; Crawford, E.M. 1965. Immunofluorescent and electron microscopic studies of polyoma virus in transformation reactions with BHK21 cells. *Exp. Mol. Pathol.* 4:51-65.

When exposed to polyoma virus in transformation experiments, BHK21 cells may be shown, by immunofluorescent tracing, to have adsorbed and ingested virus rapidly, to have retained it mostly in cytoplasmic vacuoles, and to have transmitted it to daughter cells for at least six successive mitoses. Electron microscopy and recovery of virus hemagglutinin supported these conclusions. Ingested virus was not seen in cell nuclei. Three days after infection, new virus antigen appeared in a small proportion of nuclei, increased in amount, and spread to the cytoplasm of the cells. Similar observations were made in colonies arising from individual polyoma-infected cells.

5613

Hare, J.D.; Morgan, H.R. 1964. Polyoma virus and L cell relationship: II. A curable carrier system not dependent on interferon. *J. Nat. Cancer Inst.* 33:765-775.

The persistent infection of strain L cells with polyoma virus can be characterized as follows: A high proportion of cells may carry complete, infectious virus in their cytoplasm without obvious cytopathology and may enter and complete mitosis; a low percentage of cells enter a cytolytic cycle; the infection can be cured by the cultivation of infected cells for a prolonged period in specific antiserum; interferon is not responsible for the protection of exposed cells from the cytolytic effect of polyoma virus; and a state of autologous interference may provide some protection for potentially susceptible cells.

5614

Jahkola, M.; Vainio, T. 1964. Polyoma virus and mouse strain susceptibility. *Acta Pathol. Microbiol. Scand.* 61:60-66.

Polyoma virus infection has been studied in C57B1/6 and some other mouse strains to elucidate the relative resistance of the former strain to polyoma virus oncogenesis. In in vivo experiments with a virus preparation of moderate potency, an incidence of 43 per cent of tumors could

be recorded in DBA/2 mice inoculated with virus as newborns; the incidence was less than 10 per cent in C57B1/6 mice and F-1 hybrids treated in the same way. Multiple tumors were observed only in the DBA/2 strain. In in vitro studies no differences could be established in the course of polyoma infection in embryonic fibroblast cultures of the C57B1/6 and four other mouse strains. The different possibilities for the mechanism of hereditary resistance to viral oncogenesis are discussed.

5615

Khare, G.P.; Consigli, R.A. 1965. Multiplication of polyoma virus: I. Use of selectively labeled, H3, virus to follow the course of infection. *J. Bacteriol.* 90:819-821.

Polyoma uncoating occurs in the cytoplasm at 6 hours. The nucleus is the site of polyoma synthesis. FA was used to monitor the disappearance of viral antigen between 3 and 15 hours postinfection and its reappearance at 18 to 21 hours.

5616

Kisch, A.L.; Fraser, K.B. 1964. The effect of pH on transformation of BHK21 cells by polyoma virus: I. Relationship between transformation rate and synthesis of viral antigen. *Virology* 24:186-192.

The frequency with which BHK21 hamster fibroblast cells undergo transformation after infection by polyoma virus was studied as a function of the pH at which cultures were incubated following the adsorption of virus. The transformation rate was highest at pH values between 7.6 and 7.8 and lowest at pH 7.0 and below. This phenomenon appeared to be directly related to pH rather than to bicarbonate ion concentration and to be independent of secondary selective processes. The change in the percentage of infected cells that synthesized nuclear viral antigen detectable by immunofluorescent staining was not the same as the change in the percentage of cells that underwent transformation as pH was varied.

5617

Levinthal, J.D.; Takacs, B.; Eaton, M.D. 1963. The distribution of polyoma viral antigen as detected by immunofluorescence in the tissues of infected mice. *Acta Unio Int. Contra Cancr.* 19:318-321.

Cells containing polyoma viral antigen can be detected by immunofluorescent staining of paraffin-embedded tissue sections from infected mice fixed in 95 per cent alcohol. The pathogenesis of the viral disease and its relation to the number and type of tumors produced were studied

in 80 infected mice sacrificed at intervals for the early distribution of viral lesions, and in 27 tumor-bearing mice with 49 primary tumors sacrificed for detection of later lesions. Virus was found to replicate briefly in all tissues where polyoma tumors have been observed. Chronic viral lesions occurred with large infecting doses. Viral antigen was only rarely observed in the polyoma-induced tumors.

5618

Medina, D.; Sachs, L. 1963. Studies on the lytic interaction and cell transformation with a large- and a small-plaque mutant of polyoma virus. *Virology* 19:127-139.

Quantitative studies were undertaken, with the small-plaque polyoma mutant SP2 and the large-plaque virus BP5, on lytic interaction in mouse and hamster cells and on transformation of hamster cells under various conditions. In the lytic interaction, one-step growth curves of the two viruses showed a higher total virus yield per culture for SP2 than for BP5. The higher total virus yield with SP2 was explained by a higher percentage of virus-yielding cells. Percentage of transformed clones was of the same order and degree of variation as that seen for plaque formation. Use of a virus mutant and differences resulting from cultural conditions can alter the rate of polyoma-induced cell transformation. Direct FA was employed to detect infected cells.

5619

Minowada, J. 1964. Effect of X-irradiation on DNA synthesis in polyoma virus - infected cultures: Autoradiographic studies. *Exp. Cell Res.* 33:161-175.

Autoradiographic studies using tritiated thymidine were carried out to elucidate the changes of DNA synthesis in mouse embryo cell cultures infected with polyoma virus. Measurement of DNA synthesis, as determined by the percentage of tritium-labeled cells and the grain count per nucleus, indicated the extent of DNA synthesized at the cellular level during polyoma virus infection. Immunofluorescent studies provided a measure of the presence of viral antigen in the infected cells. Combined analyses were made. The increase in DNA synthesis as a result of polyoma virus infection can be ascribed to both host cellular and viral DNA. Time curves of virus multiplication and of the increase in DNA synthesis are reported.

5620

Minowada, J. 1964. Deoxyribonucleic acid and protein syntheses in polyoma virus - infected mouse kidney cells in culture as studied by autoradiography and immunofluorescence. *Cann* 55:267-276.

DNA and protein syntheses in mouse kidney cell cultures infected with polyoma virus were studied by means of autoradiography. A combined observation, which allows immunofluorescence and autoradiography to be undertaken on the same cell, was also carried out to elucidate the relationship between viral antigen and DNA syntheses. Increase in DNA synthesis in the infected cell cultures could be attributed largely to viral DNA synthesis. Protein synthesis, as determined by the incorporation of tritiated leucine as an index, was found to occur continuously both in the infected and noninfected cell cultures. Nuclear and nucleolar incorporation of leucine into protein was greater in the infected than in the noninfected cells. A prominent nucleolus was considered to be the morphological expression of the increase in protein synthesis. Synthesis of viral DNA generally precedes synthesis of viral specific protein.

5621

Minowada, J.; Moore, G.E. 1963. In vitro studies on polyoma virus infection, with special reference to deoxyribonucleic acid synthesis as determined by autoradiography. *Cann* 54:59-70.

DNA synthesis in polyoma virus - infected mouse embryo cultures was studied by autoradiography and correlated cytological and immunofluorescent analyses. In confirmation of previous reports concerning the transformation of infected cultures, continuously growing cell populations were obtained. Nevertheless, these cell populations were found to be merely polyoma virus carriers, capable of producing parotid tumors after inoculation into newborn mice, but not transplantable as neoplastic cells. The autoradiographic results indicated that de novo synthesis of viral DNA takes place. Increased DNA synthesis in polyoma virus infection was concluded to be a primary response caused by active synthesis of viral DNA. In addition, it was evident that synthesis of host-cell DNA also continued to a certain extent.

5622

Munyon, W.; Hughes, R.; Angermann, J.; Bereczky, E.; Dmochowski, L. 1964. Studies on the effect of 5-iododeoxyuridine and rho-fluorophenylalanine on polyoma virus formation in vitro. *Cancer Res.* 24:1880-1886.

The formation of infectious polyoma virus in polyoma-infected mouse embryo cells grown in vitro is inhibited by 5-iododeoxyuridine (IUDR) added to the tissue culture medium between 4 and 15 to 22 hours after virus infection. The addition of IUDR at progressively later intervals of time up to 40 hours after infection results in the formation of correspondingly greater yields of infectious virus. Exposure of polyoma-infected mouse embryo cell cultures to rho-fluorophenylalanine from 4 to 15 hours after infection delays virus maturation by 19 hours. These results suggest that a virus-associated protein synthesis occurs during the eclipse period of polyoma virus formation. As determined by fluorescent antibody staining, synthesis of polyoma virus antigen varies with the amount of IUDR added. Uninfected mouse embryo cells exposed to IUDR for 48 hours show inhibition of cell division that is not reversible with thymidine. Such cells, following infection with polyoma virus, produce normal or greater than normal amounts of infectious polyoma virus than do control infected cells. IUDR appears therefore to inhibit the formation of polyoma virus by directly interfering with virus-initiated events and not by its effect on normal cellular processes. The cell death of polyoma-infected mouse embryo cells is not prevented by IUDR.

5623

Sheinin, R. 1964. Studies on the effect of 5-fluoro-2-deoxyuridine on polyoma T formation in mouse embryo cells. *Virology* 22:368-376.

Experiments in which FDU has been employed to inhibit DNA synthesis indicate that viral DNA is made no earlier than 30 minutes before the virion is formed in mouse embryo cells infected with polyoma virus. Viral protein was synthesized under conditions in which replication of polyoma DNA was prevented. Direct FA was used to stain polyoma T virus in mouse embryo cells. Cells with nuclear fluorescence were counted, and ratios to nonfluorescent cells were determined.

5624

Sheinin, R.; Quinn, P.A. 1965. Effect of polyoma virus on the replicative mechanism of mouse embryo cells. *Virology* 26:73-84.

TSPI polyoma virus elicits virus replication in essentially 100 per cent of mouse embryo cells infected at high input multiplicity. Using this system it has been demonstrated that polyoma virus prevents mitosis in mouse embryo cells and that it inhibits the synthesis of host cell DNA. This inhibition appears not to be due to extensive destruction of host DNA template. Direct FA was used to enumerate infectious centers by counting fluorescent nuclei in cells.

5625

Vainio, T.; Saxen, L.; Toivonen, S. 1963. The acquisition of cellular resistance to polyoma virus during embryonic differentiation. *Virology* 20:380-385.

Direct FA was used to detect polyoma virus antigen in the undifferentiated mesenchyme of embryonic mouse kidneys. None was detected in tubules or ureteric epithelium. Virus resistance increased during embryonic development.

5626

Vardosanidze, E.Sh.; Irlin, I.S. 1963. Luminescent microscopy study of polyoma virus antigen in transformed cultures of hamster embryo tissue. *Vop. Virusol.* 8:556-558. In Russian.

Hamster embryo tissue cultures that had been transformed by exposure to polyoma virus and had lost infectious virus were examined for the presence of polyoma virus antigen by FA. The antigen could not be found by the FA indirect method.

E. ROUS SARCOMA

5627

Kriukova, I.N.; Obukh, I.B. 1964. Distribution of infectious virus Rous and viral antigen in infected rat and mouse organisms. *Vop. Onkol.* 10:3:3-8. In Russian.

The localization of infectious Rous virus and viral antigen was studied by fluorescent antibody in rats and mice infected with Rous virus when in newborn. In rats with hemorrhagic disease, infectious Rous virus was detected very rarely. Viral antigen was revealed in the newly formed cyst walls, liver, and lymph nodes. Hence, Rous virus seems to multiply in rats, but its maturing takes place very rarely. Data on our recent experiments on irradiation of pieces of rabbit fibrous nodes and rat viscera and examination of affected animal sera for presence of virus-neutralizing antibody show a more complete form of integration of Rous virus with cells in rats than that in rabbits. In infected mice, viral antigen was detected very rarely.

5628

Lindberg, L.G. 1964. Studies in Rous rat sarcoma with fluorescent antibodies. *Acta Pathol. Microbiol. Scand.* 61:318-319.

In this investigation, living suspended sarcoma cells were analyzed. Specific immune serum against Rous virus (Schmidt-Ruppin strain) was prepared by immunizing adult chickens with a partly purified virus fraction either attenuated or inactivated with formalin or heat. In about 20 per cent of the RR-sarcoma cells the cell membranes contained some substance that reacts specifically to antibodies from the immunized chickens. The partly purified fraction used for immunization contained active virus. A homogenous brim of fluorescent material was seen on the entire periphery of the cells, and only exceptionally were fluorescent granules seen on the cell membrane. It is possible that the fluorescence is not related to the virus particles but to tumor-specific antigens.

5629

Martirosyan, D.M.; Shevliagin, V.Ya. 1965. Multiplication of Rous sarcoma virus in the mouse embryonic tissue. *Vop. Virusol.* 10:414-417. In Russian.

Rous sarcoma virus grew in mouse embryonic tissue in vitro. After a single infection of cell suspensions a cycle of intracellular synthesis of virus took place, and then small amounts of virus were released into the surrounding medium. Infection of monolayer cultures was less efficient. Only viral antigen was synthesized in cells, and no mature virus was released. Mature infectious virus was demonstrable in cells and cultural fluid of mouse embryonic tissue cultures during the first 24 hours after infection. Then it disappeared and could be detected again in the cultural fluid by the 20th day. Viral antigen could be found in cells in the first 4 days after infection, both strains, then it disappeared and reappeared in cell nuclei by the 7th day, Carr strain, or 14th day, Schmidt-Rupin strain. Both strains appeared in the cell cytoplasm by the 20th day.

5630

Obukh, I.B.; Kriukova, I.N. 1964. Interaction of Rous virus with mouse cells in vitro. *Vop. Virusol.* 9:538-543. In Russian.

In mouse fibroblasts infected with Rous virus, virus antigen, as assayed by FA, persisted in cells up to 48 hours. In the infectious form, the virus persisted for 24 hours. Following infection there was observed an enhancement of the growth potency of cells. The cultures were transformed after introduction of Rous sarcoma cells.

5631

Rapp, F.; Hsu, T.C. 1965. Viruses and mammalian chromosomes: IV. Replication of herpes simplex virus in diploid Chinese hamster cells. *Virology* 25:401-411.

Diploid Chinese hamster cells are less susceptible to herpes simplex virus than are primary cells derived from young rabbit kidneys. Study of the most sensitive Chinese hamster cell-virus system revealed a 12-hour latent period; at that time, intranuclear inclusion bodies and both intranuclear and intracytoplasmic virus antigen could be detected. Newly synthesized infectious virus was detected 2 hours later and continued to increase until 24 hours after inoculation of the cultures. A study of the replicative site of virus nucleic acid failed to reveal specific sites of synthesis on the chromosomes. Nevertheless, virus replication is necessary; the induction of the chromosomal aberrations previously observed in noninfective virus was unable to induce such chromosomal changes. However, the aberrations are not caused by synthesis of the viral nucleic acid at or near the broken or uncoiled areas on the chromosomes.

5632

Vogt, P.K.; Luykx, N. 1963. Observations on the surface of cells infected with Rous sarcoma virus. *Virology* 20:75-87.

Viral antigen could be demonstrated at the surface of cells infected with Rous sarcoma virus (RSV) 24 hours after infection. Fluorescent RSV antibody stained small granules of viral antigen and some of the elongated filaments extending from the cell periphery. In sparsely seeded cultures infected with an inoculum of one focus-forming unit of RSV per cell, almost all cells were stained by fluorescent RSV antibody by the 3rd day after infection. Intercellular contacts in chick fibroblast cultures were studied with the dark-field microscope. Normal fibroblasts had long cytoplasmic protrusions (termed filopodia) with which they established contacts with the glass substrate and with other cells. Infection with RSV induced rounding of the fibroblasts that was accompanied by a rupture of the filopodia. In an advanced stage of infection very few filopodia could be detected on the rounded cells. Fluorescence and electron microscopy carried out on the same cells on the 3rd and 4th days after infection revealed an identity between cell surface areas containing viral antigen and those showing viral particles.

5633

Vogt, P.K.; Rubin, H. 1963. Studies on the assay and multiplication of avian myeloblastosis virus. *Virology* 19:92-104.

Avian myeloblastosis virus (AMV) was found to cause a cellular resistance against infection with Rous sarcoma virus (RSV) in chick embryo cultures. One-step growth curve studies indicated first new virus at 24 hours after infection. Viral antigen, detectable with fluorescent antibody, appeared first at the cell surface on the 2nd day, accumulated there, and finally became detached from the cell during the later course of infection. Viral material within the cytoplasm was found less frequently and no AMV antigens were demonstrable in the nucleus. A preliminary description is given of a direct assay for AMV in tissue culture, based on counting foci of antigen-producing cells stained with fluorescent antibody.

5634

Zilber, L.; Shevlijaghyn, V. 1964. Transformation of embryonic human cells by Rous sarcoma virus. *Nature* 203:194-195.

Neoplastic transformation of rat fibroblast tissue cultures by Rous sarcoma virus was studied. Cell growth of infected cells did not vary from that of uninfected controls over a 2 month period. On the 35th day FA demonstrated viral antigen in the infected cells.

F. OTHER TUMOR VIRUSES AND GENERAL STUDIES

5635

Amano, S.; Ichikawa, Y.; Iwakata, S. 1961. Study on the relationship between malignant cell proliferation and cancer virus by means of electron microscopy and fluorescent-antibody technique. *Acta Pathol. Jap.* 11:304-305.

The causative agents of malignant tumor belong to RNA-type and are 80 to 95 nm in diameter. That of benign tumor is DNA-type and is about 40 nm.

5636

Cheville, N.F.; Olson, C. 1964. Cytology of the canine oral papilloma. *Amer. J. Pathol.* 45:849-872.

The cytologic features of the canine oral papilloma have been investigated by the techniques of histochemistry, immunofluorescence, and electron microscopy. The first tissue reaction to viral infection was hyperplasia

of keratin producing cells with no detectable virus present. Large vesicular cells appeared that contained Feulgen-positive inclusion material in the nucleus. Cells containing these inclusions fluoresced when incubated with conjugated anti-papilloma gamma globulin. There was no evidence of virus in the proliferating keratogenic cells, which were responsible for the majority of the tissue reaction. Virus particles first appeared in cells of the upper stratum spinosum. In the stratum granulosum intranuclear virus particles were seen in diffuse and in linear patterns. The formation of virus arrays as seen with electron microscope correlated with the development of basophilic inclusions seen by light microscopy, and appeared to be responsible for the specific immunofluorescence. The virus is composed of a capsid consisting of an inner core and well defined capsomeres. It is relatively stable, can be stored indefinitely in 50 per cent glycerin in saline, and it is not ether-sensitive.

5637

Friend, C.; De Harven, E.; Haddad, J. 1963. Studies on several lines of murine lymphomas associated with intracytoplasmic particles. *Acta Unio Int. Contra Cancr.* 19:344-347.

Certain lymphomas in Swiss and DBA/2 mice have been found to be associated with intracytoplasmic particles readily demonstrable by electron microscopy. Results obtained by the indirect immunofluorescence method and acridine orange staining indicated that these particles might possibly contain nucleoprotein of DNA type. Preliminary attempts to transmit these transplantable lymphomas with cell-free preparations have shown that more than 50 per cent of the treated animals developed the disease after a latent period of several months.

5638

Hampton, E.G. 1964. Viral antigen in rat embryo in culture infected with the H-1 virus isolated from transplantable human tumors: Cytochemical studies. *Cancer Res.* 24:1534-1543.

The infection of rat embryo cells with H-1 virus was followed with FA. After 12 hours the stain revealed a diffuse fluorescence that occupied the nucleus except for areas corresponding to the nucleoli. The stained nuclei increased in number during the subsequent 16, 20, and 24 hours corresponding to the log phase of viral growth. On the 3rd day after inoculation, when the viral growth curve had reached a plateau, a homogeneous type of fluorescent antibody stain was present in nuclei. Some cells contained granular or diffuse stain in the cytoplasm. At this stage acridine stain showed nuclear structural changes and homogeneous nuclei of DNA-like material. Treatment with pepsin and nucleases indicated the presence in the homogeneous nuclei of a deoxyribonucleoprotein resistant to DNAase. Phase microscopy

also showed nuclear alteration. By the 3rd day, when the viral antigen was present in most of the cells, inclusion bodies and cytopathic effect were present. These changes were followed by the release of hemagglutinating virus.

5639

Henle, G.; Henle, W. 1965. Evidence for a persistent viral infection in a cell line derived from Burkitt's lymphoma. *J. Bacteriol.* 89:252-258.

Exposure of a cell line, EB2, derived from Burkitt's lymphoma to vesicular stomatitis (VSV) and certain other viruses revealed a marked resistance of the cultures to infection. Intact, but not disintegrated, lymphoma cells induced resistance to VSV in human embryonic kidney, amnion, and diploid, as well as in green monkey kidney cells employed as feeder layers. A line of rabbit kidney or Earle strain L cells was not protected under similar conditions. Depending upon the number of EB2 cells initially transferred, resistance developed within 1 day or in more than 2 weeks. Cell-free media collected from cultures of EB2 cells in the presence or absence of feeder layers of human cells were capable of conferring rapid protection against VSV to human-cell cultures but not to L cells. The protection principle complies with present criteria for an interferon. The results are compatible with the suggestion that EB2 cultures are latently infected with a virus, as yet unidentified. FA demonstrated virus-infected tissue culture cells.

5640

Holmes, K.V.; Choppin, P.W. 1965. Cytological effects of SV5 virus. *Federation Proc.* 24:2433:571.

Effects of SV5 virus on a line of hamster kidney cells, BHK, and primary rhesus monkey kidney cells, MK, were compared. In both cells the latent period is 6 to 7 hours and the doubling time is 50 minutes. The total yield per BHK cell is only seven PFU, whereas the yield per MK cell is 500 to 1500 PFU in 24 hours and production continues for days. Development of viral antigens is similar in BHK and MK cells. Immunofluorescent perinuclear foci are seen at 3 to 4 hours; coarse and fine granules progressively appear and fill the cytoplasm by 23 hours. In BHK cells SV5 infection causes striking giant-cell formation that time-lapse photomicrography shows to be due to cell fusion. Polykaryocyte formation begins at 6 hours and involves all nuclei by 12 hours. At 17 hours the entire monolayer consists of large syncytia with up to 250 nuclei; by 36 hours the monolayer has detached. In contrast, MK monolayers develop few giant cells and remain intact for days. Large inocula induce giant-cell formation in BHK cells before viral multiplication; 6000 PFU per cell cause incorporation of all nuclei into giant cells in 1 hour, but 18,000 PFU per cell have no such effect on MK cells. The results suggest that differences in the cell membranes play a major role in determining the effects of SV5 virus on the two cell types. Complete article.

5641

Kato, S.; Miyamoto, H.; Takahashi, M.; Kamahora, J. 1963. Shope fibroma and rabbit myxoma viruses: II. Pathogenesis of fibromas in domestic rabbits. *Biken J.* 6:135-143.

The correlation among tumor formation, virus multiplication, inclusion formation and antibody response was studied in domestic rabbits inoculated with Shope fibroma virus. When 1 million rabbit infective doses per ml of virus was inoculated intracutaneously, a palpable tumor appeared on the 4th day, reaching a maximum size on about the 8th day, and disappearing completely in a month. The virus titer of the tumor gradually increased for 10 to 12 days and then rapidly decreased to zero on the 25th day. Inclusions staining reddish purple with Giemsa stain were found exclusively in the cytoplasm and were rich in viral antigen. The developmental form of the inclusions was similar to that of B type inclusions of other poxviruses, for they developed from a compact to a diffuse form, which seemed to cause degeneration of infected cells. Inclusion-bearing cells appeared and reached a maximum far earlier than did the infectivity titer or tumor formation. The percentage of inclusion-bearing cells in the total tumor cells in scratch preparations was rather low, 40 per cent at most; these decreased to zero as early as the 10th day. Complement-fixing antibody and neutralizing antibody showed a parallel increase from the 4th to 20th day. A possible mechanism of tumor formation of fibromas is discussed.

5642

Kato, S.; Takahashi, M.; Miyamoto, H.; Kamahora, J. 1963. Shope fibroma and rabbit myxoma viruses: I. Autoradiographic and cytoimmunological studies on B type inclusions. *Biken J.* 6:127-134.

The inclusions of Shope fibroma and rabbit myxoma were investigated by the fluorescent antibody technique and autoradiography with tritiated thymidine. The cytoplasmic inclusions produced in tissue culture cells and in tumor cells, with characteristics in common with B type inclusions of other poxviruses in their stainability and developmental form, were found to be the site of viral antigen and viral DNA synthesis.

5643

Kato, S.; Tsuru, K.; Miyamoto, H. 1965. Autoradiographic studies on the Yaba monkey tumor virus and host cell interaction. *Biken J.* 8:45-53.

MK2 cells infected with Yaba monkey tumor virus were studied by autoradiography with ³H-thymidine, FA, and hematoxylin-eosin staining. Inclusions found in the infected cells showed the characteristics

of 'B' type inclusions of poxviruses, being the site of viral antigen and of cytoplasmic DNA synthesis. No area that seemed to represent a secondary inclusion of poxvirus ('A' type) was found. Nuclear DNA synthesis of cells bearing 'B' type inclusions was definitely suppressed. Histologically, hematoxylinophilic inclusions, regarded as 'B' type inclusion of poxvirus, were found in the cytoplasm of many cells in the Yaba tumor. Macacus fuscatus is susceptible to Yaba monkey tumor virus.

5644

Klein, E.; Klein, G. 1964. Antigenic properties of lymphomas induced by the Moloney agent. J. Nat. Cancer. Inst. 32:547-568.

Mouse lymphomas induced by the Moloney agent were strongly antigenic in isologous hosts, as indicated by transplantation and serologic tests. Specific transplantation resistance was established by pretreatment of the recipients with homografts of other Moloney lymphomas, small isografts of the same lymphoma, or virus-containing lymphoma homogenates. Establishment of specific transplantation resistance by one of the procedures mentioned was paralleled by the appearance of humoral antibodies in the serum, detectable by the indirect fluorescent antibody reaction and by cytotoxicity in the presence of complement. The fluorescence test was specific for Moloney virus. The cytotoxic test indicated certain cross-reactions.

5645

Klein, E.; Klein, G. 1964. Mouse antibody production test for the assay of the Moloney virus. Nature 204:339-342.

Indirect FA and cytotoxic tests were used to study antibody reproduction to Moloney virus. Moloney lymphoma were good targets for indirect FA, but some were not sensitive to the cytotoxic test. Whether the antibodies detected are induced directly by the multiplying virus particles released by the tumor cells or by new cellular antigens induced by the virus infection in the host cells cannot be decided on the basis of the present evidence.

5646

Konn, M. 1963. Fluorescent antibody staining of Ehrlich ascites tumor cells. Hiroasaki Med. J. 15:746-754. In Japanese.

Antigens of Ehrlich ascites tumor cells and of mouse muscle were prepared by fluorocarbon treatment. Anti-cancer serum and anti-muscle serum were obtained from rabbits. CF test of the anti-cancer serum showed high titer of antibody against the cancer antigen but only low or undetectable antibody titer against the muscle antigen, and the anti-muscle serum

gave high titer of the homologous antibody but undetectable antibody against the cancer antigen. The complement method of FA procedure was employed for staining Ehrlich tumor cells by using the anti-cancer serum. Tumor cells were stained specifically. The staining titer of the anti-serum corresponded to the antibody titer obtained in the in vitro CF test. The antigen was limited to the cytoplasm. This procedure also stained mouse sarcoma-180 cells when the anti-cancer serum was applied, suggesting a common antigenicity between Ehrlich tumor and sarcoma-180. However, mouse leukemia SN-36 ascites cells and normal mouse ascites cells induced by the injection of nutritional broth were not stained when the anti-Ehrlich cancer serum was used.

5647

Levinthal, J.M.; Shein, H.M. 1964. Propagation of a simian tumor agent, Yaba virus, in cultures of human and simian renal cells as detected by immunofluorescence. *Virology* 23:268-270.

The immunofluorescence technique may provide a criterion for infection with Yaba virus in vitro that is more sensitive than the presence of CPE and more convenient than the use of bioassay. The use of this method permits the study of chronically infected cultures or cultures with small inocula of virus.

5648

Löffler, H. 1964. Immunofluorescence of oncogenic viruses. *Bull. Schweiz. Akad. Med. Wiss.* 20:48-53.

Oncogenic and non-oncogenic viruses can be traced in infected cells by the fluorescent antibody technique. The method works, as long as the virus material is antigenic, i.e., of protein nature. This condition is often fulfilled in virus-infected cell culture systems, but rather seldom in vivo. In tumors due to DNA virus, there seems to be a mutual exclusion of virus-producing and of proliferating cells. In RNA virus tumors, this difference of cell behavior cannot be seen.

5649

Noyes, W.F. 1965. Observations on two pox-tumor viruses. *Virology* 25:666-669.

Yaba virus and molluscum contagiosum virus were studied. Electron microscopic comparisons were made. Direct FA was used to study Yaba virus infection of Cercopithecus monkey kidney primary tissue cultures. Localization was cytoplasmic.

5650

Noyes, W.F. 1965. Studies on the human wart virus; II. Changes in primary human cell cultures. *Virology* 25:358-363.

Primary human embryonic skin and muscle cultures were inoculated with extracts obtained from pools of human wart specimens. After 2 weeks the control cultures showed orderly growth of fibroblastic cells, but the inoculated cultures showed heavy growth of randomly oriented, criss-crossed fibroblastic cells with dense focal areas composed of fibroblastic cells and piled up epithelioid cells. Electron microscopic examination of pellets of the wart extracts revealed a high concentration of typical wart virus particles with a lack of other morphological entities. The culture-altering activity of the extract was sedimented at 40,000 rpm for 1 hour and neutralized by antiserum against purified human wart virus. Transplanted, altered cultures consisted of epithelioid cells that grew in a disorganized manner. Direct FA was used to locate viral antigen in tissue sections.

5651

Osato, T.; Mirand, E.A.; Grace, J.T., Jr. 1964. Propagation and immunofluorescent investigations of Friend virus in tissue culture. *Nature* 201:52-54.

There was a relative disparity between the quantity of infectious virus, both that released and that following cell disruption, and the amount of specific immunofluorescence in the infected cells. This observation, plus the lack of significant cell surface fluorescence indicating viral release, suggests that viral antigen synthesis occurs more rapidly than maturation and release of complete virus. Previous work in the laboratory suggested that Friend disease may involve two agents: one responsible for the reticulum cell disease and the other producing the erythropoietic disorder. If this were the case, it might be possible to utilize an in vitro system such as this to identify and isolate the separate agents. Indirect FA was employed. Sequential antigen development is described.

5652

Osato, T.; Mirand, E.A.; Grace, J.T., Jr. 1965. Hemadsorption and immunofluorescence of Friend virus in cell culture. *Proc. Soc. Exp. Biol. Med.* 119:1187-1191.

In vitro propagation of Friend virus in mouse embryo tissue cultures has been observed by immunofluorescence and hemadsorption. Hemadsorption of chick erythrocytes was demonstrated. Parallel studies with immunofluorescence showed that the appearance of hemadsorption coincided

with a buildup of viral antigen at the cell surface and in the cytoplasm. Even though no gross changes were observed in the infected cells, a virus-induced modification of the infected cell surface was indicated. Hemadsorption can now be used for quantitation of the virus in culture, thus circumventing the cumbersome bioassay in mice.

5653

Rasha, A.L. 1964. Histochemical and immunofluorescent studies on Shope papillomas of cottontail rabbits. *Federation Proc.* 23:558:193.

Cytoplasmic basophilic granules and acidophilic inclusion bodies were numerous in the virus-rich and scanty in the virus-poor papillomas experimentally induced on cottontail rabbits. It became important to determine their nature and to find out whether they contained viral antigen. Like keratophyalin granules of normal skin, the papilloma granules were located in the granular layer, were Feulgen-negative and RNAase-resistant, and contained no detectable sulfhydryl groups. Acid phosphatase was not demonstrable by Barkas method, although it has been reported in normal keratophyalin by the Gomori technique. Cytoplasmic inclusion bodies were detected in all layers of the papillomas. They were Feulgen-negative, but in some the center was Feulgen-positive. They were RNAase-resistant. Acid phosphatase and -SH groups were present. Both inclusions and granules lost their affinity for toluidine blue and analine blue at the pH values at which keratin of the papilloma lost its affinity for these two dyes. No viral antigen was detected in granules or inclusions. Twenty-two different cottontail papillomas were examined; in all of them, viral antigen was found within the nuclei of the granular cells. The amount of antigen present varied from one tumor to another. Complete article.

5654

Smithies, L.K.; Olson, C. 1964. Correlation of immunofluorescence and infectivity in the developing bovine cutaneous papilloma. *Cancer Res.* 24:674-681.

Bovine papilloma antigen detected by FA was correlated with the appearance of infectious virus and with epithelial hyperplasia and hyperkeratinization in the developing bovine cutaneous papilloma. Neither antigen nor infectious virus was found in the earliest stages of growth characterized by connective tissue proliferation and by little or no epithelial change. The cellular localization of papilloma virus antigen in nuclear material of the superficial epithelium was the same when first observed as it was in the well developed wart. Regression of the warts was accompanied by a decrease in specific antigen. Marked variations in susceptibility of individual calves to bovine papilloma virus were observed, as well as differences in the histologic development of warts produced by virus administered intradermally and by scarification.

5655

Tekeli, S.; Olson, C. 1965. Distribution of an antigen in avian leukoses demonstrable by immunofluorescence. *Amer. J. Vet. Res.* 26:1442-1450.

The distribution of an antigenic material in different types of the avian leukosis complex was studied by FA, using chicken antiserum prepared against the transmissible lymphoid tumor of Olson. A specific cytoplasmic antigen was found in the lymphoid cells of transmissible lymphoid tumor, of visceral lymphomatosis produced by the Regional Poultry Laboratory isolate 12 (RPL 12) agent, and of visceral and neurolymphomatosis produced by the JM agent. Weak fluorescence was observed in the lymphoid cells of naturally occurring lymphocytoma and neurolymphomatosis, and of neurolymphomatosis associated with RPL 12 agent. Fluorescence was not observed in erythroblastosis, endothelioma, or fibrosarcoma produced by RPL 12 leukosis agent, or in Rous sarcoma. There appears to be a similar antigen in neoplastic lymphoid cells of the chicken regardless of tissue affected or whether the neoplasm occurred naturally or was experimentally produced. Absence of the antigen in neoplastic erythroblasts produced by the same virus causing lymphoid cell neoplasms indicates a basic difference between the two types of cells.

5656

Vainio, T.; Saxen, L.; Toivonen, S. 1963. Viral susceptibility and embryonic differentiation: III. Correlation between an inductive tissue interaction and the onset of viral resistance. *J. Nat. Cancer Inst.* 31:1533-1547.

Viral resistance in connection with tubulogenesis was studied. Tubules were induced in metanephrogenic mesenchyme with an artificial inducer and SE polyoma virus was applied to this system during differentiation in vitro. The distribution of this virus was demonstrated by the immunofluorescence technique. The mesenchyme that was not induced to form tubules synthesized polyoma antigen. Of the mesenchymal cells left out of the differentiation process, those enclosing the tubules seemed to support the rapid synthesis of viral protein. The epithelial cells in the tubules were not able to synthesize viral protein at any stage of infection, thus appearing resistant to polyoma virus. It was established from these experiments that the mesenchymal cells that undergo tubulogenesis fail to support the replication of polyoma virus in vitro.

5657

Vandeputte, M. 1964. Application of the immunofluorescence technique in virology and anatomic pathology. *Acta Clin. Belg.* 19:217-218. In Flemish.

The applications of the immunofluorescence technique in experimental pathology and virology are widespread. This technique was applied in the search for the viral antigen in tumors of the rat caused by the SE poliomyelitis virus and also in the tissue cultures of these tumors. With this technique the author demonstrated that although no viral antigen is present in the tumors themselves, this antigen is synthesized by the cancer cells in a certain percentage of test tube cultures of these growths. This technique also enabled him to follow the synthesis of the viral antigen morphologically on the cytologic level. A number of technical problems still have to be solved before this method becomes routine, among them, autofluorescence and non-specific fluorescence.

5658

Walter, E.L. 1964. Cellular localization of human wart virus antigen by the fluorescent antibody technique. *Federation Proc.* 23:559:194.

The significance of cytological changes associated with human warts, particularly the presence of inclusion bodies, has been given various interpretations. The present study utilizes a fluorescein-tagged rabbit antihuman wart virus antibody to locate viral antigen in acetone-fixed frozen sections of warts. Sections were stained with hematoxylin and eosin following fluorescent microscopy to correlate specific viral fluorescence with cellular morphology. Sections were also stained by the Feulgen method with and without prior DNAase treatment. Fluorescence was associated only with dark brick-red nuclear inclusions found generally at the periphery of the nucleus of the cell. Neither inclusions nor fluorescence nor DNAase-resistant DNA was found in the prickle-cell or basal cell layers. The viral antigen is not present in the epidermal cells until they approach the surface of the skin, and it is associated with dark staining nuclear inclusion bodies. These results appear to substantiate the findings of Almeida et al. with the electron microscope. Complete article.

5659

Walter, E.L., Jr.; Walker, D.L.; Cooper, G.A. 1965. Localization of specific antigen in human warts. Arch. Pathol. 79:419-423.

Fluorescein-labeled antihuman wart antiserum was prepared. This antiserum stained antigen in human warts and did not stain sections of other hyperkeratotic lesions or tissues rich in Forssman antigen. Fluorescence was quenched by unlabeled antiserum and fluorescein-labeled normal serum failed to produce fluorescence in wart sections. The histology and cytology of the human wart as seen in hematoxylin and eosin stained sections was correlated with distribution of specific wart antigen as located by fluorescence microscopy. Viral antigen was concentrated mainly in reddish-purple colored masses in the nuclear area of cells of the granular layer and in nuclear residues in the keratinized layer. Observations made on sections treated with DNAase and then stained by the Feulgen method showed that the masses of antigen contained DNA that apparently was viral DNA.

IV. HERPESVIRUS

5660

Albrecht, P.; Blaskovic, D.; Jakubik, J.; Lesso, J. 1963. Demonstration of pseudorabies virus in chick embryo cell cultures and infected animals by the fluorescent antibody technique. *Acta Virol.* 7:289-296.

Chick embryo cell (CEC) cultures were inoculated with two strains of pseudorabies virus at high and low multiplicities of infection. At a high multiplicity the incubation period was less than 3 hours and was followed by a steep rise in the virus titer. About 90 per cent of the seed virus was adsorbed onto the cells within 2 hours. The infection of CEC cultures with pseudorabies virus caused characteristic cellular lesions, but without the production of specific intranuclear inclusions or syncytium formation. Viral antigen, demonstrated by the fluorescent antibody method, was produced in excess, first in the nucleus and then in the cytoplasm, where it formed coarse granules located mostly in the paranuclear (Golgi) region. In experimentally infected rabbits and weaned pigs, scattered nerve and glial cells showed specific fluorescence; a predilection of pseudorabies virus for the ependymal lining and the periventricular nervous tissue was evident.

5661

Aurelian, L.; Roizman, B. 1964. The host range of herpes simplex virus interferon, viral DNA, and antigen synthesis in abortive infection of dog kidney cells. *Virology* 22:452-461.

Herpes simplex virus strain MP (HSV-MP) and pseudorabies virus (PSV) multiply in Hep-2 cells. In dog kidney cells PSV multiplies, but HSV-MP fails to multiply or to produce plaques. HSV-MP interferes with the multiplication of PSV in Hep-2 cells and in dog kidney cells. Studies of dog kidney cells exposed to HSV-MP revealed that these cells produce viral antigen, interferon, and small amounts of DNA characteristic of HSV-MP, but do not form particles with physical characteristics of HSV-MP. They show signs of infection but fail to multiply. Direct FA was used to detect viral antigen.

5662

Benda, R. 1965. Demonstration of B virus (Herpesvirus simiae) in HeLa cells by the fluorescent antibody technique. *Acta Virol.* 9:172-179.

The indirect fluorescent antibody technique was applied to the demonstration of B virus in cell cultures and to the detection of antibodies in sera with high titers. The kinetics of the accumulation of antigen in HeLa cells infected with large and small amounts of B virus were

studied in parallel with those of the multiplication of infectious virus and development of the cytopathic effect. The sporadic findings of inclusion bodies and differences observed in the detection of antigen by immunofluorescence in cells infected with B virus and with herpes simplex virus are discussed.

5663

Braune, M.O. 1964. Standardization and application of the fluorescent antibody technique in the diagnosis of avian viral respiratory diseases. Diss. Abstr. 24:4914-4915.

FA techniques were developed and standardized for study of the viruses of Newcastle disease, infectious bronchitis, and infectious laryngotracheitis. The conditions for preparation of conjugates and staining are given. Virus was demonstrated in cell cultures. Comparison of the relative diagnostic efficiency of FA and virus isolation indicated that FA was more sensitive in the early disease stages. The methods were equivalent at the peak of the disease, and virus isolation was better near the end. Field diagnoses were successfully attempted by FA.

5664

Braune, M.O.; Gentry, R.F. 1965. Standardization of the fluorescent antibody technique for the detection of avian respiratory viruses. Avian Dis. 9:535-545.

Each phase of the FA technique was investigated. Gamma globulin solutions prepared from specific antisera against Newcastle disease virus, infectious bronchitis virus, and infectious laryngotracheitis virus having neutralization indices of 3.5 or greater were satisfactory for conjugation. The optimum conjugation ratio of FITC to protein was 0.03 mg dye per mg protein at a conjugation time of 12 hours. Reactions of a high fluorescent intensity were observed in infected tracheal smear preparations that were fixed in acetone at -20 C for 10 minutes and reacted for 30 minutes with conjugates adjusted to contain twice the protein concentration of the FA titration endpoint. A washing time of 20 minutes or more in phosphate-buffered saline did not affect the intensity of specific reactions. Infected tracheas could be stored at 4 C under moist conditions for 72 hours and retain their reactivity.

5665

Christian, R.T.; Ludovici, P.P. 1965. Programming of virus replication within herpes simplex plaques. *Bacteriol. Proc.* V54:106.

We have reported that primary human amnion cells infected with herpes simplex virus form plaques in liquid medium and that the infected cells line the periphery of the plaque in bands roughly in order of their infection. This microepidemic was studied for DNA synthesis, viral protein synthesis, and competent virus production, by tritiated thymidine 3rd-hydrogen-labeled, fluorescent antibody, and one-step growth curve techniques, respectively. The radioactive label appeared in the infected cells approximately 2 hours after infection, FA after 4 hours, and competent virus was recovered 6 to 8 hours after infection. There was still some tritiated thymidine uptake 48 hours after infection. The uptake of tritiated thymidine for cellular DNA synthesis obscured the early stages of plaque formation, and attempts to eliminate this background with cold thymidine and metabolic inhibitors failed. However, 5,000-r X-irradiation blocked cellular DNA synthesis but allowed thymidine uptake, FA, and cytopathology in the cells within plaques. This phenomenon has been shown in amnion cells and human foreskin fibroblasts and is now under investigation in other cell strains.

5666

Dayan, A.D.; Morgan, H.G.; Hope-Stone, H.F.; Boucher, B.J. 1964. Disseminated herpes zoster in the reticuloses. *Amer. J. Roentgenol.* 92:116-123.

Four cases of generalized herpes zoster in patients suffering from a reticulosis are described. In two cases the herpes zoster was fatal and in one of them a full postmortem examination was performed. There was unusually extensive involvement of the posterior root ganglia by herpes zoster virus. The virus was identified by immunologic tests performed on tissue extracts and by a new fluorescent antibody technique applicable to tissue sections.

5667

Geder, L.; Koller, M.; Gonczol, E.; Jeney, E.; Gonczol, I. 1963. Experiments for the isolation of the herpes zoster virus. *Orv. Hetilap* 104:2031-2034. In Hungarian.

Six virus strains were isolated from the vesicle fluid of eight herpes zoster patients. Identification of the virus was proved on the basis of the cytopathogenic changes observed on fibroblast cultures, of the eosinophilic intranuclear inclusions, of the cell-bound nature of the infection, and of the neutralization tests carried out with the patients' acute and convalescent sera and the demonstration of virus within the cell by means of the immunofluorescence method.

5668

Geder, L.; Koller, M.; Gonczol, E.; Jeney, E.; Gonczol, I. 1963. Isolation of herpes zoster virus strains. *Acta Microbiol. Acad. Sci. Hung.* 10:155-161.

Six strains of virus have been isolated from vesicle fluids obtained from eight patients with herpes zoster. The cytopathic lesions observed in unstained human fibroblast cultures, the intranuclear eosinophilic inclusions, the exclusive intracellular localization of the infectious virus, the neutralization tests carried out with paired sera of the patients having yielded virus, and the demonstration of virus antigen in the infected cells with convalescent sera by the FA technique suggested the identity of the strains with the herpes zoster virus.

5669

Griffin, J.W. 1963. Fluorescent antibody study of herpes simplex virus lesions and recurrent aphthae. *Oral Surg.* 16:945-952.

Fluorescent antibody methods are reliable for the diagnosis of herpes simplex virus infections. Papanicolaou staining of smears from clinically suspicious herpes simplex infections is an excellent method for the diagnosis of these lesions when they are clinically differentiated from herpes zoster and varicella infections. Recurrent herpes simplex infection does occur within the oral cavity proper. Cells from recurrent aphthae do not exhibit specific fluorescence to labeled herpes simplex convalescent serum or to labeled human globulin obtained from a convalescent patient with recurrent aphthae.

5670

Griffin, J.W. 1965. Recurrent intraoral herpes simplex virus infection. *Oral Surg.* 19:209-213.

Four cases of intraoral recurrent herpes simplex virus disease have been presented. The cause of the lesions was verified by routine cytologic and fluorescent antibody techniques.

5671

Gurvich, E.B.; Roihel, V.M. 1965. Use of the fluorescent antibody technique in the detection and differential diagnosis of smallpox. *Acta Virol.* 9:165-171.

Studies on variola, alastrim, vaccinia, cowpox, varicella, herpes zoster, and herpes simplex viruses were carried out by the direct fluorescent antibody technique. Antigen of variola virus could be detected 6 hours after infection of human embryo cell cultures with 1,000 tissue

culture mean infective doses of the virus. With smaller doses of virus, the time periods for virus detection increased. With 1 and 0.5 mean infective doses, the viral antigen was detected in a part of the inoculated cultures after 3 days. Some peculiarities in the intracellular distribution of fluorescence caused by the presence of different viral antigens were observed. An antigenic relationship between the varicella and herpes viruses was demonstrated. Cross-reactions with homologous and heterologous globulins revealed the possibility of serological identification of viruses of the pox group as well as of their differentiation from the varicella and herpes viruses.

5672

Hayashi, K. 1965. Immunofluorescent studies on the herpes simplex infection of eyes: I. Course of the infection of rabbit eyes and the effect of corticosteroid. Jap. J. Exp. Med. 35:49P.

In the conjunctival discharges of steroid group, the specific fluorescence was first observed 3 hours after the inoculation of virus. Then it increased both quantitatively and qualitatively, reaching maximum about 48 hours after inoculation. After 96 hours the fluorescence gradually decreased and after 9 days disappeared completely. In the control group, conjunctival discharges were stained 20 hours after the inoculation and the fluorescence was recognized until the 11th day. In the sections of eyes of the control group, specific fluorescence was observed in the epithelial cells of bulbar conjunctiva, corneal epithelium, corneal stroma, epithelial cells of lacrimal glands, Harder's glands, and hair roots of eyelids. In the steroid group, it appeared in the corresponding tissues earlier than in the control group and in the epithelial cells of iris, ciliary body, and choroid. Fluorescence remained in corneal epithelium 2 days longer in the control group than in the steroid group. In all the other tissues it continued to be positive for a longer period in the steroid group than in the control group.

5673

Holmes, A.W.; Caldwell, R.G.; Dedmon, R.E.; Deinhardt, F. 1964. Isolation and characterization of a new herpes virus. J. Immunol. 92:602-610.

A new virus has been isolated from marmosets dying of generalized disease. This agent satisfies the chemical, physical, biologic, and immunologic criteria for inclusion in the group of herpes viruses. The reservoir of this virus in nature is not known but it is not likely to be the marmoset. The temporary designation of herpes M or Herpesvirus tamarinus is suggested for this agent.

5674

Husain, M.H.; Sommerville, R.G. 1964. Presence of herpes simplex virus on eczematous skin. *Lancet* 2:391-392.

The frequent presence of herpes simplex virus on eczematous skin has been demonstrated. Its possible role in exacerbating the eczema is briefly discussed.

5675

Johnson, R.T. 1964. The pathogenesis of herpes virus encephalitis: I. Virus pathways to the nervous system of suckling mice demonstrated by fluorescent antibody staining. *J. Exp. Med.* 119:343-356.

The pathogenesis of herpes simplex virus encephalitis and myelitis was studied in suckling mice, using routine titration procedures and fluorescent antibody staining for the identification of infected cells. After intracerebral inoculation, virus dispersed rapidly in the cerebrospinal fluid multiplied in meninges and ependyma, and then invaded the underlying parenchyma to infect both neurons and glia. Following extraneural inoculation, virus gained access to the central nervous system (CNS) by both hematogenous and neural pathways. After intraperitoneal and intranasal inoculation virus multiplied in viscera and produced viremia; foci of CNS infection then developed around small cerebral vessels. After subcutaneous and intranasal inoculation, neural spread of virus was demonstrated along corresponding peripheral and cranial nerves. This spread resulted from the centripetal infection of endoneural cells, Schwann cells, and fibroblasts. Antigen was not found in axons even after infection of the corresponding ganglion cell perikaryon. Subsequent spread within the CNS was unrelated to neural tracts, and there was no evidence of axonal spread of virus in the host-virus system studied.

5676

Johnson, R.T. 1964. The pathogenesis of herpes virus encephalitis: II. A cellular basis for the development of resistance with age. *J. Exp. Med.* 120:359-374.

The resistance to herpes virus encephalitis that develops with age was studied in mice by fluorescent antibody staining. Adult mice remained susceptible to intracerebral inoculation, and the infection of the central nervous system was identical to that found in immature mice. A barrier to the spread of virus inoculated extraneurally developed with maturation, and the limitation of spread appeared to coincide with the infection of peritoneal and tissue macrophages. In vitro, suckling and adult mouse macrophages were infected with equal ease.

However, suckling mouse macrophages infected other cells in contact with them, but infected adult mouse macrophages did not. Studies failed to reveal the nature of this change in macrophages, which developed with age. The role of macrophages in the pathogenesis of herpes virus encephalitis is discussed. The hypothesis is made that an alteration in the macrophages of the maturing mouse plays an important role in its development of resistance to herpes virus encephalitis.

5677

Koller, M.; Gonczol, E.; Vaczi, L. 1963. Study of the multiplication of varicella-zoster virus by the fluorescent antibody test. *Acta Microbiol. Acad. Sci. Hung.* 10:183-188.

The pathological changes and the intracellular location of viral antigen have been studied in human embryonic fibroblast cultures at different times following inoculation with varicella-zoster virus. The first cytopathic changes were visible 10 hours after inoculation. At this time some minute, eosinophilic granules, each surrounded by a light area, appeared. Characteristic Type A inclusions were visible 48 to 72 hours after inoculation. Complete destruction of cells took 96 to 144 hours. Viral antigen was first detectable about the 10th hours of infection. Minute fluorescent spots were seen in the nuclei. In the period from 24 to 48 hours nuclear fluorescence increased and cytoplasmic fluorescence appeared. After the 72nd hour the antigen gradually disappeared from the nucleus but not the cytoplasm. The intracellular distribution of viral antigen and the formation of Type A nuclear inclusions seem to be parallel phenomena.

5678

McAllister, R.M.; Straw, R.M.; Filbert, J.E.; Goodheart, C.R. 1963. Human cytomegalovirus: Cytochemical observations of intracellular lesion development correlated with viral synthesis and release. *Virology* 19:521-531.

The evolution of the intracytoplasmic and intranuclear lesions produced by a human cytomegalovirus in human embryo cells is described and the lesions are characterized cytochemically. Twenty-four hours after infection, basophilic, lipid-containing, PAS-positive bodies surrounded by a halo containing RNA appear in a juxtannuclear position in the cytoplasm. Forty-eight hours after infection amphophilic patches that contain DNA and viral antigen appear in the nucleus. The cytoplasmic lesion also contains viral antigen at that time. Seventy-two hours after infection the cytoplasmic lesion contains DNA and the nucleus is significantly enlarged. At about this time, infectious virus is first found intracellularly, and shortly thereafter release into the medium begins. Indirect FA was used to detect infected cells.

5679

Mihail, A.; Aderca, I. 1963. Detection of herpes antigen in cell culture by means of fluorescent antibodies. Stud. Cercet. Inframicrobiol. 14:435-440. In Rumanian.

The authors investigated the presence of herpes virus antigen in the cell by the direct fluorescent antibody technique. Three herpes virus strains were cultivated in human embryo cell and human amniotic cell cultures, line FL. The fluorescent immune serum was human gamma globulin coupled with fluorescein isothiocyanate or with lissamine rhodamine B 200. Within 18 to 24 hours after inoculation, the appearance of isolated cells or small cellular islets, with fluorescent nuclei, was observed. After 36 to 48 hours both nuclear and cytoplasmic fluorescence were noted in the cellular islets, as well as cells with fluorescent cytoplasm and a dark, apparently empty nucleus. The results demonstrate that the herpes antigen initially appears in the nucleus, then passes into the cytoplasm. No difference was noted in the localization of the herpes antigen for the three virus strains investigated.

5680

Mohanty, S.B. 1964. Studies on indirect hemagglutination and immuno-fluorescence with infectious bronchitis virus. Diss. Abstr. 24:4921-4922.

An improved indirect hemagglutination (IHA) test using formalinized tannic acid treated sheep erythrocytes (FTC) was developed for detecting infectious bronchitis virus (IBV) antibodies in chicken serum. The growth of the virus in chicken embryo kidney cell cultures was studied by FA. Factors in the IHA test are detailed. The virus (Beaudette) produced the syncytial type of cytopathogenic effect in chicken embryo kidney cell cultures. The viral antigen could not be detected by the direct FA one and 3 hours postinoculation. Specific nuclear staining was observed after 7 hours. Twenty-four hours after inoculation, the entire nucleus exhibited a bright granular fluorescence with some release of viral antigen. At 36 hours, release of antigen was detected in the cytoplasm, and the virus entered a second cycle of multiplication. Flazo orange masked nonspecific fluorescence in tissue culture preparations.

5681

Mohanty, S.B.; DeVolt, H.M.; Faber, J.E. 1964. A fluorescent antibody study of infectious bronchitis virus. *Poultry Sci.* 43:179-182.

Growth of IBV in chicken embryo kidney cell cultures was studied by FA. Viral antigen appeared first in the nucleus at 7 hours and later in the cytoplasm. The entire nucleus contained a granular fluorescence at 24 hours. Some antigen was released at that time. By 36 hours antigen was detected in the cytoplasm. A second virus multiplication began at that time. Flazo orange was a successful counterstain.

5682

Munk, K.; Fischer, H. 1965. Immunofluorescent differences in herpes simplex virus strains. *Arch. Ges. Virusforsch.* 15:539-548. In German.

For various herpes simplex strains, differences in appearance and in the localization of immunofluorescence in the host cells could be observed 24 hours after infection. Virus strains with the same cytopathic and plaque characteristics also show the same immunofluorescence characteristics. Thus the classification of the virus species herpes simplex into biologically distinct groups can also be confirmed by FA tracing.

5683

Nii, S.; Kamahora, J. 1963. Location of herpetic viral antigen in interphase cells. *Biken J.* 6:145-154.

Studies were made on the appearance of herpetic viral antigen in monolayer cells in vitro. The plus-GC and minus-GCr variants of the Miyama strain and Yamagishi strain virus were used. A high multiplicity of virus was inoculated onto FL or L cell monolayers. Approximately 4 hours after virus inoculation, fluorescent material appeared at the nuclear membrane and in the perinuclear regions of the cytoplasm. In the early stages of infection the fluorescent granules were very fine. In the nucleus, fluorescence could not be detected. The antigen spread extensively in the cytoplasm. In experiments with the minus-GCr type of virus, the infected cells became round, and in later stages it was difficult to decide the exact site of the antigen in cells. Nuclei in the syncytium produced by plus-GC virus did not seem to have fluorescent material. Syncytial formation induced by a high dose of plus-GC virus was distinct as early as one hour after virus inoculation, which was before the antigen began to appear in the cells. The viral antigen was detected earlier than the formation of typical intranuclear inclusion bodies.

5684

Pettit, T.H.; Kimura, S.J.; Peters, H. 1964. The fluorescent antibody technique in diagnosis of herpes simplex keratitis. Arch. Ophthalmol. 72:86-98.

An evaluation of this technique as a diagnostic tool for clinical herpetic keratitis is far from completed. The good correlation between the ability to isolate herpes simplex virus in tissue culture and to obtain a positive identification by the fluorescent antibody is confirmed. The reliability of the test in cases that are clearly herpes keratitis clinically is demonstrated. The importance of developing a clinically useful viral diagnostic test had become more apparent with the advent of antiviral agents. The rapidity and ease with which the fluorescent antibody test can be carried out in laboratories where facilities are available give it considerable advantage over the more laborious and time-consuming techniques of virus isolation by culture.

5685

Pettit, T.H.; Kimura, S.J.; Uchida, Y.; Peters, H. 1965. Herpes simplex uveitis: An experimental study with the fluorescein-labeled antibody technique. Invest. Ophthalmol. 4:349-357.

Direct FA was used to study inoculation of herpes virus into one eye of a rabbit. Ten to 14 days later uveitis developed in the opposite uninoculated eye. With FA the virus could be demonstrated in the cells of the iris, ciliary body, corneal endothelium, and trabecular meshwork of the inoculated eye as long as 7 days after inoculation. From the 4th to the 8th day after inoculation the virus could be traced spreading along the posterior ciliary nerves of the inoculated eye toward the central nervous system. On the 9th to 15th day, FA staining demonstrated the presence of the virus proliferating in the retina but sparing the choroid of the uninoculated opposite eye. Careful sectioning of both optic nerves and the chiasm from the 2nd through the 19th day after inoculation showed patchy viral involvement of the nerves and chiasm. An unsuccessful search was made for nerve spread. If the virus was inoculated into the vitreous cavity of the right eye, it could be found in the retina of that eye in 48 hours and followed as a progressive infection of the glial cells along the right optic nerve to the chiasm. Infection of the left retina, however, appeared at 9 days after inoculation and before passage of the virus up the left optic nerve could be demonstrated. The inability to demonstrate a continuous spread of the virus along the nerves to the opposite eye suggest an intermittent viremia.

5686

Rado, J.P.; Tako, J.; Geder, L.; Jeney, E. 1965. Herpes zoster house epidemic in steroid-treated patients: A clinical and viral study. Arch. Intern. Med. 116:329-335.

In a house epidemic of herpes zoster, four of the six affected patients were receiving corticosteroid treatment and one suffered from acute arsenic poisoning. The patient who was the source of infection never received steroid treatment. Generalized herpes zoster developed in two patients during corticosteroid therapy. The average incubation period was 18 days. A cytopathogenic agent was successfully isolated from vesicular material in a patient treated with prednisolone, and was identified as a zoster-varicella virus by neutralization tests and fluorescent antibody studies. Specific antibody formation was normal in three of the four examined patients treated with steroids.

5687

Rado, J.; Tako, J.; Geder, L.; Jeney, E.; Gonczol, I. 1964. Succession of herpes zoster cases in patients under corticosteroid treatment. Orv. Hetilap 105:1266-1270. In Hungarian.

Four of six patients affected by the outbreak of a house epidemic of herpes zoster had received corticosteroid treatment; one had arsenic poisoning. The patient who was the source of the infection had never received steroids. Two patients on corticosteroids developed generalized herpes zoster. The average incubation time was 18 days. A cytopathogenic agent isolated from the vesicle fluid of a patient on prednisolone was identified as zoster-varicella virus by neutralization and immunofluorescence tests. Specific antibody production was normal in three of the four examined cases. The outbreak of the epidemic was attributed to exogenous virus infection, susceptibility to which was increased by corticosteroid therapy. Increased susceptibility to the virus was not caused by impaired antibody production due to the influence of corticosteroids.

5688

Rapp, F. 1964. Replication of herpes zoster virus in human embryonic lung cells and inhibition by metabolic analogues. Federation Proc. 23:862:244.

The replication and spread of herpes zoster virus in cultures of human embryonic lung cells was studied in the presence and absence of iododeoxyuridine, IUDR, and cytosine arabinoside. Adsorption of infected cells to fresh monolayers was complete within 15 minutes. The cultures were maintained in the absence and presence of IUDR and harvested at various intervals for the detection of cells capable of giving rise

to plaques and for detection of virus antigens by immunofluorescence. Eight to 16 hours were required for the virus to infect neighboring cells. Maximum yields were obtained 40 to 48 hours after inoculation of the cultures. Spread of the virus was accompanied by cytopathic changes typical of zoster infection. Plaque formation by zoster-infected cells was almost completely inhibited by 10 gamma per ml of either IUDR or cytosine arabinoside. Although IUDR prevented spread of zoster virus from cell to cell, as much as 100 gamma of the analogue per ml did not cure infected cultures. Complete article.

5689

Rapp, F.; Hsu, T.C. 1965. Viruses and mammalian chromosomes: IV. Replication of herpes simplex virus in diploid Chinese hamster cells. *Virology* 25:401-411.

Diploid Chinese hamster cells are less susceptible to herpes simplex virus than are primary cells derived from young rabbit kidneys. Study of the most sensitive Chinese hamster cell-virus system revealed a 12-hour latent period; at that time, intranuclear inclusion bodies and both intranuclear and intracytoplasmic virus antigen could be detected. Newly synthesized infectious virus was detected 2 hours later and continued to increase until 24 hours after inoculation of the cultures. A study of the replicative site of virus nucleic acid failed to reveal specific sites of synthesis on the chromosomes. Nevertheless, virus replication is necessary; the induction of the chromosomal aberrations previously observed in noninfective virus was unable to induce such chromosomal changes. However, the aberrations are not caused by synthesis of the viral nucleic acid at or near the broken or uncoiled areas on the chromosomes.

5690

Rapp, F.; Rasmussen, L.E.; Benyesh-Melnick, M. 1963. The immunofluorescent focus technique in studying the replication of cytomegalovirus. *J. Immunol.* 91:709-719.

A quantitative assay was developed for cytomegalovirus involving counts of immunofluorescent foci of antigen-containing cells. Counts were made 6 to 7 days after infection. The number of foci seen decreased with decreasing concentrations of virus and a linear relationship was obtained over the countable range. Comparable virus titers were obtained in parallel immunofluorescent focus and tube titrations, but the tube titration was slow and required about 30 days of observation. The focus method was used to study virus adsorption and replication in embryonic human lung fibroblasts. Adsorption proceeded rapidly at 37 C; 90 per cent of the virus adsorbed in 1 hour. Adsorption proceeded more slowly as the temperature was decreased. At 37 C pro-

duction of virus antigen and maturation of virus required 4 days after inoculation of the cells, although cellular changes could be observed earlier if the cultures were fixed and stained. The infection subsequently spread to adjacent cells and increasing numbers of cells comprised the infected foci.

5691

Rapp, F.; Vanderslice, D. 1964. Spread of zoster virus in human embryonic lung cells and the inhibitory effect of iododeoxyuridine. *Virology* 22:321-330.

Zoster virus infects human embryonic lung cells, but subsequently no free virus appears in the culture. When seeded onto cellular monolayers, infected cells produce plaques under agar. A linear relationship has been established between the number of infected cells seeded and the number of plaques that develop. The spread of zoster virus has been followed by calculating the production of plaque-forming cells and correlating this with immunofluorescent detection of zoster antigen. Eight to 16 hours were required for the virus to infect neighboring cells. The virus then spread from cell to cell throughout the culture; maximum infection was obtained 40 to 48 hours after inoculation. This spread was accompanied by cytopathic changes typical of zoster infection. Iododeoxyuridine prevents spread of zoster virus from cell to cell, but does not eradicate the infection because virus antigen and plaque-forming cells can be detected as long as 5 days after inoculation of the cultures in the presence of 100 ug of iododeoxyuridine per milliliter.

5692

Schmidt, N.J.; Lennette, E.H.; Woodie, J.D.; Ho, H.H. 1965. Immunofluorescent staining in the laboratory diagnosis of varicella-zoster virus infections. *J. Lab. Clin. Med.* 66:403-412.

The use of immunofluorescent staining techniques in the identification of varicella-zoster (V-Z) virus isolates and in the serologic diagnosis of V-Z virus infections is described. Identification of viral isolates was facilitated through the use of a specific immune serum prepared in monkeys; immune globulins were conjugated with fluorescein isothiocyanate for use in direct fluorescent antibody staining. A comparison was made of the value of indirect FA staining and complement fixation tests for serologic diagnosis of V-Z virus infections, and the two techniques were found to be comparable in their ability to demonstrate diagnostically significant antibody titer rises. Serologic diagnosis by these two techniques was equally reliable in patients with clinical diagnoses of chickenpox and in those with diagnoses of herpes zoster.

5693

Siminoff, P. 1964. The effect of 5-bromodeoxyuridine on herpes simplex infection of HeLa cells. *Virology* 24:1-12.

At 0.00001 M bromodeoxyuridine (BDU) inhibited the production of infectious virus by HeLa cells maintained in suspension or monolayer cultures. The drug-sensitive reaction appeared to begin about 2 hours after infection, preceding the earliest appearance of progeny virus by 2 hours. When added within 2 hours after exposure of infected cells to BDU, thymidine restored virus productivity; thereafter, cells progressively lost the capacity to produce infectious virus. Infected cells treated with BDU produced intranuclear DNA-containing inclusions, formed polykaryocytes, and accumulated viral antigen in a pattern of virus-induced cytopathology indistinguishable from that of the normal infection. Infection of BDU-treated cells that had lost the ability to divide resulted in a normal yield of infectious virus. Viral antigen was detected by direct FA.

5694

Smith, K.O.; Dukes, C.D. 1964. Effects of 5-iodo-2-desoxyuridine (IDU) on herpesvirus synthesis and survival in infected cells. *J. Immunol.* 92:550-554.

Treatment of cells with IDU immediately following a 2-hour period of virus adsorption completely suppressed the production of new infectious virus but did not prevent the synthesis of new viral components. Both fluorescent and electron microscopy revealed the formation of large amounts of viral material in drug-treated, infected cells, although most input infectious virus disappeared. Ragged, naked, structurally imperfect particles were produced in the presence of IDU. It appeared, therefore, that the antiviral activity of IDU was due to an interruption in the assembly of viral components, not in their production.

5695

Sydiskis, R.J.; Schultz, I. 1965. Herpes simplex skin infection in mice. *J. Infect. Dis.* 115:237-246.

The growth curve of the virus during the first 27 hours after injection could be related to that in tissue culture. The amount of virus in the lesion increased to a peak at the 5th day and then gradually diminished. The local progression of virus within the lesion was demonstrated by fluorescent antibody staining methods. Specific fluorescence was localized only in cells of epidermal origin after the 2nd day, and the histopathology of the lesion showed degeneration of epidermal cells. The similarities between this mouse lesion and human herpes simplex dermatitis suggest that it may serve as a model of this infection in man.

5696

Tanaka, S.; Southam, C.M. 1965. Zoster-like lesions from herpes simplex virus in newborn rats. *Proc. Soc. Exp. Biol. Med.* 120:56-59.

Newborn rats are susceptible to infection and death by herpes simplex virus administered by many routes. When the virus is admitted intradermally a segmental skin eruption resembling herpes zoster develops. Hepatitis and encephalitis also occur. The etiologic role of herpes simplex virus in these skin and visceral lesions was demonstrated by neutralization and fluorescent antibody studies.

5697

Uchida, Y.; Kimura, S.J. 1965. Fluorescent antibody localization of herpes simplex virus in the conjunctiva. *Arch. Ophthalmol.* 73:413-419.

FA was used to demonstrate specific localization of herpes simplex antigen in the conjunctiva of rabbits with experimental herpetic keratoconjunctivitis. Viral localization was noted in the cytoplasm as well as in the nuclei of conjunctival epithelial cells. Corneal infection with herpes simplex virus was not necessary for infection of the conjunctival epithelium. Clinically, the experimental conjunctival lesions did not form a dendritic pattern or vesicles; histologically they were small, superficial, plaque-like, and sparsely distributed. They were identified more easily by fluorescent antibody staining than by staining with hematoxylin and eosin.

5698

Vaczi, L.; Gonczol, E.; Lehel, F.; Ceder, L. 1965. Isolation of cytomegalovirus and incidence of complement-fixing antibodies against cytomegalovirus in different age-groups. *Acta Microbiol. Acad. Sci. Hung.* 12:115-121.

A group of 16 symptom-free premature babies and infants with clinically demonstrated cytomegalic inclusion disease or interstitial pneumonia has been examined. Urine samples from four infants yielded cytopathogenic agents in human embryonic fibroblast cell cultures. On the basis of their growth characteristics, cytopathogenicity, neutralization, and FA tests, the agents appeared to be cytomegaloviruses (CMV). The properties of the strains are described. In spite of failing serology, the typical clinical picture and the successful isolations suggested the possible etiological role of the isolated agents. The incidence of CF antibodies against CMV was found to increase with age in serum samples obtained from a total of 442 persons of different ages. Incidence and titers of CF antibodies against CMV in the sera of 60 mentally defective children corresponded to data from healthy children.

5699

Yamamoto, T.; Otani, S.; Shiraki, H. 1965. A study of the evolution of viral infection in experimental herpes simplex encephalitis and rabies by means of fluorescent antibody. *Acta Neuropathol.* 5:288-306.

The routes of access of herpes simplex virus into the suckling mouse central nervous system are hematogenous dissemination that affects mainly the cerebrum and cerebellum, and a neural pathway by the spinal cord and the autonomic system. The main hosts in herpetic infection of the nervous systems are neurones, astrocytes, and Schwann cells. Most of the viral antigen is confined to the cytoplasm. The most common visceral sites for herpes virus multiplication are the intestine and lung. The host in rabies infection is the nerve cell. Virus antigen is distributed in both peripheral processes and cytoplasm. In the ganglia of both cranial and spinal nerves, brain stem, and diencephalon, rabies virus antigen appears at an earlier stage of infection and is larger in amount than in the cerebrum and cerebellum. Fine particles of rabies virus antigen in acinar cells of the salivary gland first appear 3 days after infection, gradually aggregate in the cellular periphery around the acinar space, and finally agglomerate into coarse granules located in the acinar lumina 9 days after inoculation.

V. ADENOVIRUS

5700

Carmichael, L.E. 1964. The pathogenesis of ocular lesions of infectious canine hepatitis: 1. Pathology and virological observations. Pathol. Vet. 1:73-95.

Disease-free dogs, known to be susceptible to infectious canine hepatitis, were inoculated with this virus subcutaneously or intravenously. Antibody and virus titers were measured in the aqueous humor and blood serum. Ocular tissue was examined for the presence of virus by FA. During the acute febrile stage of illness, there was mild iridocyclitis without corneal involvement. The latter supervened during convalescence. Two fundamentally different modes of pathogenesis are postulated for the ocular lesions of infectious canine hepatitis. During the acute phase of illness, prior to the onset of circulating antibody, uveitis occurs that is related directly to effects of viral growth in vascular endothelium, and in reticuloendothelial elements of the anterior uvea. The severe iridocyclitis with corneal opacification that occurs in clinically recovered dogs has characteristics of the Arthus-type of ocular hypersensitivity.

5701

Carmichael, L.E. 1965. An incomplete cycle of adenovirus Type 4 multiplication in canine cell cultures and in dogs. Amer. J. Vet. Res. 26:15-23.

By immunofluorescence, autoradiography, and conventional staining methods it was shown that deoxyribonucleic acid and viral antigen were synthesized in canine cell cultures after inoculation with adenovirus Type 4. The multiplication cycle of this virus in canine cells was incomplete, however, as infectious virus was not produced. By immunodiffusion and complement-fixation tests, viral antigen was found to be primarily cell-associated. Antigens produced in dog kidney cells infected with adenovirus Type 4 differed from those produced in HeLa cells. Similar results were obtained in dogs inoculated with adenovirus Type 4. Viral antigen was found by FA in regional lymph nodes. However, virus infectious for HeLa cells was not recovered.

5702

Carmichael, L.E. 1965. The pathogenesis of ocular lesions of infectious canine hepatitis: II. Experimental ocular hypersensitivity produced by the virus. *Pathol. Vet.* 2:344-359.

The pathogenesis of the ocular lesions that occur during convalescence from infectious canine hepatitis was studied in susceptible dogs by passive sensitization, reverse-passive sensitization, and by intra-ocular inoculation with virus-antibody precipitate suspensions. Iridocyclitis and corneal opacification could be produced. These lesions resembled natural ones. The natural ocular lesions that follow infectious canine hepatitis are a consequence of Arthus-type hypersensitivity reactions.

5703

Clemmer, D.I. 1964. Characterization of agents isolated from market chickens in a quest for enteric viruses. *J. Infect. Dis.* 114:386-400.

Thirty-four agents were isolated from the cloacas of young chickens. Two isolates were studied. They produced adenovirus-like CPE and were ether-resistant. Neither agglutinated chick red cells or was antigenically related to several other well-characterized avian viruses. Small series of normal sera from humans, monkeys, calves, guinea pigs, and rabbits contained no neutralizing antibody to either agent. Strain 65 was antigenically related to the viruses of GAL Types 1 and 2 and Strain 93 to GAL Types 3 and 4 and to EV-89. Strain 93 was inactivated by formaldehyde and by heating at 80 C, but not 70 C. It did not agglutinate sheep red cells but agglutinated rat red cells at 37 C. A marked increase in nuclear deoxyribonucleic acid content was present in chick kidney cells infected with Strain 93 and stained at 20 hours by Feulgen and acridine orange techniques. An accumulation of intranuclear ribonucleic acid was also observed. By immunofluorescent staining the viral antigen was visualized in particulate form within the nucleus. No evidence of virus was observed in the nucleolus or cytoplasm.

5704

Connor, J.C.; Marti, A. 1964. Use of primary human cell cultures in titrating certain human adenoviruses for infectivity. *Proc. Soc. Exp. Biol. Med.* 117:38-45.

Adequate measurement of infectivity of human adenovirus Types 3 and 12 was found to depend upon time of incubation and upon the type of in vitro cell culture system employed. Primary human amnion cells were found to be superior to human fibroblastic cells derived from

skin of fetuses and to two established cell lines, HeLa and KB, for infectivity titrations of Type 3 and Type 12 viruses. Direct FA was used to detect adenovirus in the nuclei of tissue culture cells.

5705

Geck, P.; Dan, P.; Nasz, I. 1964. Examination of the cytopathic effect of adenoviruses by immunofluorescence. *Acta Microbiol. Acad. Sci. Hung.* 11:19-22.

Types 3 and 5 of adenovirus characterized by different types of cytopathic effect have been studied by FA in primary human amnion cell cultures. It has been shown that the structures appearing in the cell after virus infection had the same antigenic character as the virus. The data obtained suggested that virus is formed as early as in 7 to 10 hours following infection. An asynchronism in the onset of virus multiplication was also observable. In the final stage, however, all cells exhibited specific fluorescence.

5706

Geck, P.; Imre, G.; Korchmaros, I.; Nasz, I.; Dan, P. 1964. Specific antigen properties of keratoconjunctivitis epidemica inclusions: Tests using the immunofluorescent method. *Orv. Hetilap* 105:439-441. In Hungarian.

A keratoconjunctivitis epidemic occurred in Hungary in 1961 to 1962. Characteristic inclusion bodies were found in the epithelial cytoplasm of 88 per cent of the scrapings taken during the acute phase of the disease. Through immunofluorescent tests it was established that these inclusion bodies have specific antigen properties which conform to the antigenic nature of type 8 adenovirus, the main pathogenic agent in this epidemic. According to this, the inclusions of keratoconjunctivitis epidemica partly or entirely contain the pathogenic virus particles.

5707

Gocke, D.J.; Preisig, R.; Morris, T.Q.; Sweeting, J.G.; Bradley, S.E. 1965. Correlation of viral invasion with hepatic dysfunction in experimental canine hepatitis. *J. Clin. Invest.* 44:1051.

Beagle puppies were infected parenterally with 300 TCID₅₀ of virus. Daily hepatic biopsies, routine biochemical tests, serologic studies, and determinations of Bromsulphalein (BSP) storage capacity (S) and transport maximum (Tm) were carried out. Dogs without antiviral antibody died after 5 to 7 days. Viremia was demonstrable by day 2 to 3, associated with fever and accumulation of inflammatory cells in hepatic sinusoids and virus in Kupffer cells. Subsequently, foci of hepatocellular necrosis containing virus developed, and elevations of SGOT appeared.

BSP S and Tm were decreased from controls. The Tm/S ratio remained normal. This depression was proportional to cellular destruction. At autopsy extensive hepatic necrosis was present. Dogs with low levels of antibody had a longer course. Small necrotic foci containing virus were found in the liver after 3 to 4 days, with minimal functional derangement. Lesions appeared to regress, but after 2 to 3 weeks, extensive hepatic necrosis developed together with inflammatory infiltrates in portal areas and around central veins. Virus was no longer demonstrable in the liver. S and Tm decreased markedly. Functional abnormalities can be related to viral invasion and extent of hepatic damage. Immunity modulates the disease.

5708

Goulet, N.R.; Schriver, P.W.; Liu, O.C.; Beiler, J.M. 1965. Isolation and propagation of adenoviruses in African green monkey kidney cultures. J. Infect. Dis. 115:429-435.

Evidence is presented that unlimited growth of adenoviruses in African green monkey kidney cultures can be obtained without the presence of SV40. Virus growth appears to be based on the selection of a mutant highly adapted to monkey kidney cultures after preliminary passage in human cell cultures. FA was used to demonstrate adenovirus in both African green monkey kidney and human embryonic kidney tissue cultures.

5709

Imre, G.; Korchmaros, I.; Geck, P. 1963. Demonstration of the inclusion bodies in epidemic keratoconjunctivitis with the immunofluorescence method. Szemeszet 99:25-28. In Hungarian.

The immunofluorescence method was used for demonstrating the inclusion bodies in epidemic keratoconjunctivitis. Because of the lack of specific immune serum, fluorochrome was linked to the globulin fraction of convalescent serum. Those smears that showed inclusion bodies when stained with Giemsa displayed vividly fluorescent spots in the protoplasm of the treated cells. Their number, shape, size, and location conform to those of the inclusion bodies. The specificity of the stain proves that the bodies are antigens and that the convalescent serum of patients with epidemic keratoconjunctivitis contains corresponding antibodies. These investigations seem to support the belief that the inclusion bodies are identical with viral colonies.

5710

Ketiladze, E.S.; Zhilina, N.N.; Mekler, L.B.; Naumova, V.K.; Lozhkina, A.N.; Orlova, N.N.; Nisevich, L.L. 1964. Use of the fluorescent antibody method for rapid differential diagnosis of influenza, parainfluenza, and adenovirus illnesses. Vop. Virusol. 9:348-353. In Russian.

The direct immunofluorescence method with highly purified fluorescent antisera against influenza, parainfluenza, and adenoviruses was employed to detect virus antigen in cells of nasopharyngeal mucosa of patients with acute respiratory illnesses. The preparations were stained in parallel with acridine orange fluorochrome for revealing RNA and DNA inclusions. During an inter-epidemic period the method proved helpful for determining virus etiology of more than 50 per cent of such cases within the first days of illness. A good correlation was found between the results of immunofluorescence and those of serological investigations with paired sera. RNA and DNA inclusions were demonstrable more often in cases where positive immunofluorescence with a respective serum was seen. During a small influenza B outbreak positive immunofluorescence results with anti-influenza serum were obtained in 75 per cent of patients with influenza and early influenza complications. This is a valuable and highly specific method for early differential diagnosis of acute respiratory virus infections.

5711

Ketiladze, E.S.; Zhilina, N.N.; Mekler, L.B.; Naumova, V.K.; Orlova, N.N.; Lozhkina, A.N.; Nisevich, L.L. 1964. The significance of the fluorescent antibodies method for rapid etiological diagnosis of acute respiratory viral affections in children. Vop. Okhrany Materin. Detstva 9:8:28-33. In Russian.

Results are presented of direct FA method used in examining the nasopharyngeal smears obtained from 120 children with acute respiratory diseases. The use of fluorescent sera containing antibodies against influenza virus, parainfluenza virus, and adenoviruses has made possible identification of 56 per cent of the infections, 14 per cent of which proved to be etiologically connected with influenza, 19 per cent with parainfluenza, and 23 per cent with adenoviral infection. The viral antigen was detectable at any stage of the disease when marked clinical symptoms of the disease were present. Comparison of the immunofluorescence results on the smears and serological and virological investigations demonstrated not only the high specificity of FA, but also ascertained its definite diagnostic advantages. The rapidity and the simplicity of the direct FA method has offered a possibility of obtaining the results of nasopharyngeal smear examinations in 3 hours. This makes this method most promising for clinical practice.

5712

Kishko, Ya.H.; Pervachenko, S.V.; Nosach, L.M.; Mykhailova, E.H.; Vantsak, N.P. 1965. A fluorescent study of adenoviruses of Types 3 and 5 in a tissue culture of cancer cells. *Mikrobiol. Zh.* 27:1:5-10. In Russian.

Cultures of cancer cells, normal and infected by adenoviruses of Types 3 and 5, were treated by Feulgen's method, at various periods after inoculation with the infectious material, with acridine orange, DNAase, and by indirect FA. The best method for this purpose is fixation of the preparations with ethyl alcohol at room temperature. After preliminary treatment by DNAase, the Feulgen test and staining with acridine orange make possible the detection of adenovirus DNA after 12 hours. By that time and at later periods indirect FA could be used for reliable differentiation by type-specific sera of Types 3 and 5 adenovirus.

5713

Magureanu, E.; Musetescu, M.; Grobnicu, M. 1965. Histological study of adenovirus Type 14 development in cell cultures. *J. Hyg.* 63:99-104.

By means of the fluorescent antibody method and acridine orange staining, experimental infection with adenovirus Type 14 has been investigated in KB and MK cell cultures. Intracellular changes could be shown to precede the appearance of the cytopathic effect. Thus, progressive stages in the development of adenovirus at this level could be separated both by the demonstration of the specific antigen and by the appearance of an altered DNA. Both methods have proved to be equally useful for the detection of virus multiplication and of the histochemical changes induced in the cells. No significant differences have been found to exist with respect to the cell line used.

5714

Mayor, H.D. 1964. A labile intranuclear RNA associated with the development of adenoviruses. *J. Exp. Med.* 119:433-442.

The nucleic acids produced intracellularly during the replication cycles of both DNA and RNA viruses can now be identified rapidly by a sensitized procedure based on staining with the fluorochrome acridine orange. Cellular DNA, viral DNA, cellular RNA, and RNA arising as a result of viral stimulus can be differentiated. The intracellular development of virus-specific DNA, RNA, and protein has been studied in monkey kidney cells infected with adenoviruses Types 3 and 7. It has been possible to detect a labile RNA in the nucleus from 16 to 20 hours after inoculation. When the cultures are treated with

puromycin at this time, this RNA can be accumulated under certain conditions in the nucleus and demonstrated cytochemically. At the same time, the production of specific viral protein as determined by staining with fluorescein-labeled antibodies is markedly inhibited. However, intranuclear double-stranded DNA continues to be formed for a time. When puromycin is added to the system early in the eclipse period virus-specific DNA and labile RNA cannot be detected.

5715

Mayor, H.D.; Jamison, R.M.; Jordan, L.E.; Melnick, J.L. 1965. Structure and composition of a small particle prepared from a simian adenovirus. *J. Bacteriol.* 90:235-242.

When tissue-culture fluids infected with simian adenovirus SV15 are examined in an electron microscope, either as fresh harvests or after treatment with Genetron, typical mature adenovirus particles are found. These are 65 to 70 mu in diameter, with an icosahedral capsid built from 252 capsomeres. Also present is a population of small polyhedral particles approximately 20 mu in diameter. These small particles can be separated from the mature virions by ultrafiltration or density gradient centrifugation. The small particles have a density of 1.43 in cesium chloride. They contain protein and double-stranded deoxyribonucleic acid. They appear to possess cubic symmetry of the icosahedral type, with a coat composed of 12 subunits, each at the vertex of an icosahedron. Antigenic nature of the particle was determined by FA.

5716

Moulton, J.E.; Frazier, L.M. 1964. Multiplication of canine hepatitis virus in the presence of 5-fluorodeoxyuridine. *Proc. Soc. Exp. Biol. Med.* 115:970-976.

5-Fluorodeoxyuridine (FUDR) was only partially effective in suppressing synthesis of canine hepatitis virus in prolonged cultures of dog kidney cells. Suppression was more effective when cultures were inoculated with large amounts of virus than with small amounts. Examinations of cover slip cultures by acridine orange, by fluorescent antibody, and by microspectrophotometry of material stained by the Feulgen method revealed DNA and virus in a few cells in each FUDR-inhibited culture. Usually there was no increase in the mean level of DNA per inhibited culture. It was concluded that the multiplication of canine hepatitis virus in the presence of FUDR occurred in a few resistant cells in each culture and after successive cycles of infection the titers became as high as in virus controls.

5717

Moulton, J.E.; Frazier, L.M. 1964. Cytopathic changes in tissue cultures inoculated with infectious canine hepatitis virus and treated with 5-fluorodeoxyuridine. *Amer. J. Vet. Res.* 25:41-46.

Acridine orange staining of dog kidney cells inoculated with infectious canine hepatitis virus showed increase in nuclear deoxyribonucleic acid in 12 to 18 hours. Intranuclear inclusion bodies containing DNA developed in 18 hours and increased in number during the later stages of infection. Viral synthesis at sites of DNA increase was indicated by specific immunofluorescence. In contrast, when 5-fluorodeoxyuridine (FUDR) was added to the cultures before or shortly after the virus, cellular changes failed to develop until the 42nd hour, at which time there were only a few changes. When the cultures were treated with FUDR at varying times after addition of virus, the later the addition of FUDR, the more severe were the cytopathic changes. Thymidine added to infected cultures at the same time as FUDR caused partial reversal of the inhibitory effect of FUDR.

5718

Moulton, J.E.; Frazier, L.M. 1965. Early nuclear changes in cells infected with canine hepatitis virus. *Amer. J. Vet. Res.* 26:723-726.

Acridine orange and FA methods were used to study the changes that appear in dog kidney cells during the early stages of infection with canine hepatitis virus. DNA synthesis occurred in the nucleus 8 to 10 hours after infection and preceded viral protein accumulation by approximately 2 hours. Antigenic viral protein was found in the cytoplasm at the time it first appeared in the nucleus.

5719

Nosik, N.N. 1964. A study of succinic dehydrogenase activity in adenovirus Type 5 - infected tissue cultures. *Vop. Virusol.* 9:455-459. In Russian.

Cytochemical, cytological, and biological tests have revealed a significant diminution of succinic dehydrogenase activity in susceptible HeLa and SCH cell lines infected with adenovirus Type 5. This diminution is first noted 24 hours after infection and then it proceeds in parallel to the increase of virus in cell cultures. No alteration in the enzyme activity has been observed in L cells in which adenovirus does not multiply. These data suggest that this enzyme takes no part in the synthesis of a virus particle and its suppression at comparatively late periods of the infectious process is the result of degenerative changes of the cell caused by virus.

5720

Nuzzolo, L.; Ravetta, M.; Vellucci, A. 1963. Report of immunologic relationships of the adenovirus group studied by immunofluorescence. *Nuovi. Ann. Ig. Microbiol.* 14:44-55. In Italian.

Immunological relationships among adenoviruses Types 7, 11, and 14 have been observed by indirect fluorescent antibody technique. HeLa, KB, and monkey kidney cells were used for the research.

5721

Salenstedt, C.R. 1963. Studies on the virus of hepatitis contagiosa canis (HCC): V. A study with the aid of fluorescent antibodies on some features of HCC virus infection in the dog and guinea pig. *Acta Vet. Scand.* 4:384-394.

A statistical analysis of the difference in the number of fluorescent cells between sections from different parts of the tonsil was made. There was no significant difference between sections from the same small piece, but when the sections originated from different parts of the tonsil significant differences were obtained in some cases. A constant ratio between 'fluorescence titer' and virus titer could not be established. The role of the tonsils and small intestinal wall as portal of entry of infection in dogs was studied. Virus multiplied in epithelial cells of these loci. Viral antigen was also present in cells of phagocytic nature, in endothelial and in lymphoid cells. HCC antigen appeared in cells on the surface of the liver and spleen as well as in the peritoneal exudate in guinea pigs. A rapid method of diagnosing HCC is described. In spontaneously or experimentally infected dogs cell material was scraped from the tonsillar surface. After staining with a fluorescent anti-HCC serum the occurrence of viral antigen could be shown in cells in the collected specimens.

5722

Tobe, T. 1965. Inapparent virus infection as a trigger of a. endicitis. *Lancet* 1:1343-1346.

During the past 5 years 642 appendices were removed and examined microscopically. Lymphoid hyperplasia in various degrees was found in 91 per cent of the 474 infected cases. Many sera of patients with appendicitis showed raised complement fixation titers against Coxsackie-B virus or adenovirus. With a fluorescent antibody technique, Coxsackie-B virus and adenovirus were clearly demonstrated in the cytoplasm of histiocytes adjacent to the epithelium of the mucous membrane of the appendix, in the cytoplasm of reticulum cells in the lymph follicles of the appendix, and in the cytoplasm of hyperplastic mesenteric lymph nodes in many cases. Tissue cultures have so far been negative. Inapparent virus infection plays an important part as a trigger of appendicitis.

5723

Voronina, F.V.; Pille, E.R. 1963. A study of multiplication of simian viruses in tissue culture by fluorescent antibody method. Vop. Virusol. 8:594-600. In Russian.

The distribution in cultured monkey kidney cells of three simian viruses of different groups was studied by immunofluorescence. The accumulation of antigen in cells was compared with the development of the cytopathic effect in acridine orange stained preparations and with the accumulation of virus in the cultural fluid. The antigen of the virus of the first groups, which is similar to adenoviruses, accumulated in the nucleus in DNA-containing grains and dense inclusion bodies; at later stages the virus antigen appeared in the cytoplasm. Antigens of the viruses of the second and third groups, which are similar to enteroviruses, are localized only in the cytoplasm. A parallelism is noted between the accumulation of antigen in cells and the increase of virus titer in the cultural fluid. Differences in the distribution and time of appearance of antigens of the three virus groups are described.

5724

Zalmanzon, E.S.; Liapunova, E.A. 1963. The effect of 5-fluorouracil on the synthesis of Type 5 adenovirus nucleic acid and protein. Acta Virol. 7:481-489.

5-Fluorouracil in a concentration of 25 micrograms per ml lowered the reproduction of Type 5 adenovirus and the synthesis of its protein to 0.1 to 8.0 per cent of the control. Removal of 5-fluorouracil from the culture after 20 hours' contact with the infected cells did not restore the ability of adenovirus to multiply. 5-Fluorouracil did not affect the multiplication of virus in the presence of equimolar amounts of thymidine. The inhibitory action of 5-fluorouracil on virus multiplication was less pronounced in the presence of thymine or cytidine. Equimolar amounts of uracil or uridine did not influence the inhibitory effect of 5-fluorouracil, but 10 times higher concentrations of uracil or uridine were effective. After 20 hours of contact of 5-fluorouracil with the infected cells, the inhibitory effect of the analog could be completely reverted by thymidine and partially also by equimolar amounts of thymine. Amethopterin suppressed completely the multiplication of Type 5 adenovirus. 5-Fluorouracil inhibited the synthesis of Type 5 adenovirus DNA. FA conjugates prepared from rabbit antisera were used to detect virus antigen.

VI. RABIESVIRUS

5725

Allen, R.; Sims, R.A.; Sulkin, S.E. 1963. Persistence of rabies virus in cultured bat brown adipose tissue. Texas Rep. Biol. Med. 21:448.

The interscapular brown adipose tissue of the bat can be grown in explant and monolayer culture. Evidence of rabies virus multiplication in cultured bat brown fat was obtained 3 days after inoculation with 6,000 mouse intracerebral LD₅₀ and persisted for at least 56 days at 37.5 C. Extracellular virus was quantitated by intracerebral inoculation of culture fluids into mice, and cell-associated virus was best demonstrated by FA. Rabies antigen was located intracytoplasmically and occurred both diffusely and in discrete aggregations. When FA preparations were stained with May-Grunwald-Giemsa, the discrete areas of specific immunofluorescence stained as blue inclusion bodies surrounded by halos. Throughout the course of the experiment no evidence of cell degeneration was observed despite the appearance of inclusions and evidence of virus multiplication. These studies support the theory that the interscapular brown adipose tissue of the bat is capable of sustaining latent rabies infection and contributing to the ability of this animal to act as a natural reservoir for this agent. Complete article.

5726

Allen, R.; Sims, R.A.; Sulkin, S.E. 1964. Studies with cultured brown adipose tissue: I. Persistence of rabies virus in bat brown fat. Amer. J. Hyg. 80:11-24.

The interscapular brown adipose tissue of the bat has been cultivated and found to support the growth of rabies virus in the following manner: (a) an adsorption period of 7 hours is required to achieve infection of the maximum number of cells; (b) although virus antigen can be detected in a small percentage of cells 24 hours following inoculation, cultures do not reach peak infectivity values until 15 to 20 days after inoculation; (c) cell-associated virus is best demonstrated by FA and appears both as diffuse fluorescence and as discrete aggregations throughout the cytoplasm; (d) when FA preparations are stained with May-Grunwald-Giemsa, aggregations of specific immunofluorescence appear as blue inclusion bodies surrounded by halos; (e) rabies infection has been shown to persist in cultured bat brown fat for at least 56 days at 37.5 C with no gross degenerative changes observed. These studies support the hypothesis that the interscapular brown adipose tissue of the bat is capable of sustaining rabies infection and contributing to the ability of this animal to act as a natural reservoir for this agent.

5727

Allen, R.; Sims, R.A.; Sulkin, S.E. 1964. Studies with cultured brown adipose tissue: II. Influence of low temperature on rabies virus infection in bat brown fat. *Amer. J. Hyg.* 80:25-32.

The multiplication of rabies virus in cultures of bat brown fat is suppressed by low temperature as evidenced by failure to demonstrate more than trace amounts of virus in fluids from infected cultures after transfer to 8 C. Although no conclusive evidence of virus multiplication at low temperature was obtained, the presence of rabies virus antigen in cold cultures was made visible by the fluorescent antibody technique. That viable virus persisted in infected brown fat cells held for varying periods at 8 C was shown by transferring cultures back to 37.5 C, whereupon fluid phase virus rapidly reached demonstrable levels, and cells exhibited brilliant immunofluorescence.

5728

Askew, J.B.; Marks, B.E.; Leach, R.E. 1965. International Border rabies. *J. Amer. Med. Women's Ass.* 20:464-466.

A rabies epidemic in Tijuana is described. One aspect discussed was use of fluorescent rabies antibody testing. The test is described.

5729

Atanasiu, P.; Lepine, P.; Dragonas, P. 1963. Kinetics of rabies virus in tissue cultures, with reference to fluorescent antibodies and electron microscope study of ultrathin sections. *Ann. Inst. Pasteur Paris* 105:813-824. In French.

Two strains of rabies virus, street virus and the fixed Pasteur strains, have been studied on two cell lines. The street virus slowly propagates on BHK 21-C13 cells and reaches its maximal virulence on the 12th day. The cells show a partial autolysis with severe metabolic disturbances. Rabies antigen appears at the level of the nuclear wall at the 15th hour after infection of the cell. It afterwards reaches the cytoplasm and the cell wall. The supernatants contain the maximal amount of virus. It was possible to titrate the fixed virus by fluorescent antibody. Of the cell lines BHK 21-C13 and Ep.0, the latter seems to be the more sensitive. By precipitation in gel medium and immunoelectrophoretic analysis, the authors have demonstrated the presence of two soluble antigens in the BHK 21-C13 cells infected with both strains of rabies virus. Electron microscope observations were reported.

5730

Baratawidjaja, R.K.; Morrissey, L.P.; Labzoffsky, N.A. 1965. Demonstration of vaccinia, lymphocytic choriomeningitis and rabies viruses in the leucocytes of experimentally infected animals. Arch. Ges. Virusforsch. 17:271-279.

During the stage of viremia, viral antigen was demonstrable by means of immunofluorescence in the leukocytes of peripheral blood of experimental animals infected with vaccinia, lymphocytic choriomeningitis, and rabies viruses. Specificity of the fluorescence observed was confirmed in each case by re-isolation of the virus from washed leukocytes by animal or tissue culture inoculation and the identity of re-isolated virus was established by neutralization or immunofluorescence techniques. Possibility of employing FA for early diagnosis of viral infections is discussed.

5731

Beauregard, M.; Boulanger, P.; Webster, W.A. 1965. The use of fluorescent antibody staining in the diagnosis of rabies. Can. J. Comp. Med. Vet. Sci. 29:141-147.

Brain material from 750 domestic and wild animals submitted to this laboratory for rabies diagnosis was studied by the following three methods: (1) microscopic examination of Williams' stained impressions, (2) mouse inoculation test, and (3) microscopic examination of impressions stained by FA. The purpose of this investigation was to compare the sensitivity of FA with that of two classical methods. From the results obtained by one or the other method of study, 175 specimens were diagnosed as positive. Of these, only 33 per cent were detected by the examination of Williams' stained impressions. On the other hand, two rabid cases were missed by the mouse inoculation test, and four by FA. Without being completely reliable, the last two methods proved to be almost equally sensitive and much more so than the examination of Williams' stained impressions.

5732

Campillo-Sainz, C. 1964. The use of fluorescent antibodies in the study of some infectious diseases: III. The immunofluorescence technique in the study of rabies. Gac. Med. Mex. 94:315-317. In Spanish.

Use of FA for rabies diagnosis overcomes disadvantages of Negri body examination and mouse inoculation. FA may be used on nervous tissue and in salivary glands. Use of the latter for the diagnosis of rabies is of particular importance. Another advantage of the technique is that it can be carried out in less than 5 hours. Besides the intensely

fluorescent Negri corpuscles, in positive preparations numerous smaller luminous points are observed that are called 'antigenic dust.' The specificity of the technique is similar or even better than the intracerebral inoculation of mice. FA demonstrated that the Negri corpuscle is made up of conglomerates of viral particles. BA-46-95149.

5733

Clark, H.F.; Shepard, C.C. 1963. A dialysis technique for preparing fluorescent antibody. *Virology* 20:642-644.

A dialysis method is described for conjugating serum proteins with FITC. Antisera against rabies, polio, rickettsiae, and mycobacteria were used. Human, monkey, guinea pig, sheep, and rabbit globulins were conjugated by this method. Good specific FA staining with minimal nonspecific staining was obtained.

5734

Crandell, R.A. 1965. Laboratory investigation of arctic strains of rabies virus. *Acta Pathol. Microbiol. Scand.* 63:587-596.

Viruses identified as rabies by the demonstration of negri bodies in experimental animals, by immunofluorescence, and by serum-virus neutralization tests were isolated from animals from Greenland. In comparing several laboratory diagnostic methods for detection of the virus, the fluorescent antibody technique and mouse inoculation method were shown to be equally sensitive. Rabbits, guinea pigs, white mice, and hamsters were susceptible to infection by different routes of inoculation. Hamsters were found to be more susceptible than white mice for primary isolation. Rabbits were more refractory than the other species studied. In addition to various types of cytoplasmic inclusion bodies, intranuclear inclusions were demonstrated in nerve cells of brains of some laboratory-infected animals.

5735

Dean, D.J.; Evans, W.M.; McClure, R.C. 1963. Pathogenesis of rabies. *Bull. WHO* 29:803-811.

Spread of fixed and street strains of rabies virus from the site of injection to the central nervous system and salivary glands in various animal species was studied. The results indicate conclusively that rabies virus is ordinarily transmitted from the site of exposure to the central nervous system via the peripheral nerves but that other than nerve transmission may occur in young animals, in highly susceptible species, or in animals whose resistance has been altered by trauma or shock. Airborne infection is occasionally possible. Blood-

borne infection in nature is believed to be exceptional and less likely to occur in man, whose resistance to rabies is high, than in animals of species known to be highly susceptible. Evidence of nerve-borne transmission was also observed with herpes simplex virus but not with lymphocytic choriomeningitis virus of the GD7 and FA strains of mouse encephalomyelitis virus.

5736

Fernandes, M.V.; Wiktor, T.J.; Koprowski, H. 1963. Mechanism of the cytopathic effect of rabies virus in tissue culture. *Virology* 21:128-131.

Following exposure to rabies virus, infected cells of the BHK-21, HDCS, and RE cultures continue to divide, displaying the presence of viral antigen continuously in their cytoplasm. Large inclusion bodies accumulate in the cytoplasm of the progeny of originally infected cells after they have undergone several divisions. The presence of the large inclusion prevents mitosis and eventually leads to cell lysis. This indirect cytopathic mechanism observed in BHK-21 and HDCS cultures may be characteristic of fixed rabies virus infection, making impossible the utilization of these culture systems for quantitative determination of rabies virus. On the other hand, kinetics of rabies infection in these cell systems may be different from that observed in the RE tissue culture system, where cytopathic effect was not observed.

5737

Fernandes, M.V.; Wiktor, T.J.; Koprowski, H. 1964. Endosymbiotic relationship between animal viruses and host cells: A study of rabies virus in tissue culture. *J. Exp. Med.* 120:1099-1116.

Rabbit endothelial cell (RE) cultures infected with a fixed rabies virus were studied. The virus can propagate itself in these cells for an indefinite period of time without interfering with cell growth. Virus-specific antigen was detected in the cytoplasm of each cell by fluorescein-labeled antirabies serum. Only 4 to 5 per cent of the cells released infectious virus. Cells undergoing division showed viral antigen. Growth rate, plating efficiency, and morphological characteristics of both infected and control cultures were identical. No difference was detected between RE and rabies-infected RE cultures (RE-CVS) cell population by RNA and DNA labeling experiments. Antirabies serum effectively inhibited the spread of extracellular virus. It did not interfere with cell-to-cell transmission of the virus during mitosis. RE-CVS cells, when exposed to fresh antirabies serum lysed completely, but inactivated serum had no lytic effect. Addition of hamster complement to the inactivated serum restored its cytolytic properties. The serially passaged RE-CVS virus gradually became less virulent for mice and displayed a weak antigenicity in the mouse protection test. Another feature of the RE-CVS cell system is its resistance to infection with homologous and heterologous viruses despite the apparent absence of an interferon-like substance.

5738

Frederickson, L.E. 1963. Rabies in an endemic area and the value of the fluorescent antibody technique. J. Tennessee Med. Ass. 56:311-313.

For many years Tennessee had one of the highest rabies rates in the country. Intensive efforts toward the vaccination of dogs has reduced the rate in these animals to a remarkable degree. The high rate among foxes continues. The use of FA technique in examination of tissue from animals has simplified diagnosis, made it more accurate, and spared many people from antirabies vaccination.

5739

Girard, K.F.; Hitchcock, H.B.; Edsall, G.; MacCready, R.A. 1965. Rabies in bats in southern New England. New Engl. J. Med. 272:75-80.

Brain tissue of 520 bats of various species were examined by Sellers staining and FA for rabies. Eight were positive, confirmed by animal inoculation. Five of these displayed no signs of illness. Rabies virus was found in the urine of three of these bats. The positive bats were collected in eastern Massachusetts only. These findings, coupled with studies on ranges of the species of bats found positive, indicate the need for close epidemiologic observation of these geographic areas.

5740

Gispen, R.; Saathof, B. 1965. Neutralizing and fluorescent antibody response in man after antirabies treatment with suckling rabbit brain vaccine. Arch. Ges. Virusforsch. 15:377-386.

A group of 18 persons were treated with UV-irradiated and lyophilized antirabies vaccine, derived from brain tissue of 4- to 5-day-old suckling rabbits (SRE vaccine) and devoid of encephalomyelitic activity in guinea pigs. Serological responses following the postexposure treatment were measured by neutralization and indirect immunofluorescence methods. Prevaccination sera were negative. The FA test demonstrated early antibody response within 13 to 17 days after the first vaccine injection in all subjects. The neutralization test demonstrated 9 of 11 early antibody responses. The ratio of reactors increased to 13/13 during the interval between early and late samples. A group of 18 persons was treated with a modification of live attenuated Hoegyes-Phillips vaccine derived from adult rabbit brain. None had rabies antibodies before the immunization. All 18 tested by FA and all 13 tested by neutralization reacted positively. The median titers found by both methods were, however, about one-fourth that of the SRB group. Indirect FA appeared to be a convenient and rapid method for titrating rabies antibodies; it seemed to be more sensitive than neutralization. Local and other benign secondary reactions after treatment with each or both vaccines were not different. No reactions of the nervous system occurred.

5741

Gustafson, A.A.; Hundley, J.B. 1962. Fluorescence microscopy: A new diagnostic tool in North Dakota. Proc. N. Dakota Acad. Sci. 16:75-78.

The method has greatly facilitated diagnosis in streptococcal cases but has not been sufficiently tested for rabies and enteropathic Escherichia coli. BA-44-7351.

5742

Hannoun, C.; Fernandes, M.V.; Macieira-Coelho, A. 1965. Effects of cortisone on in vitro viral infection: I. Inhibition of cytopathogenesis by polio, rabies, and yellow fever viruses in human embryonic cells. Proc. Soc. Exp. Biol. Med. 119:153-158.

Plaque formation, observation of cytopathic effect, immunofluorescence, and mouse inoculation were used variously to evaluate some aspects of cortisone acetate on infection of diploid human embryonic fibroblastic cultures with polio, rabies, and yellow fever viruses. Cortisone did not affect poliovirus directly, but did inhibit plaque production when cells were subjected to many passages in its presence. Both accumulation of cytopathic effect and spread of virus in cultures infected with rabies virus before serial subculture were inhibited by cortisone in passage medium. Cortisone exerted a similar cumulative effect on virus production by cells infected with yellow fever virus, and an immediate inhibition of cytopathic response, but both virus production and cytopathic effect returned on removal of cortisone from the medium. The findings suggested a cumulative but nonselective effect of cortisone on cellular capacity to respond to virus infection.

5743

Johnson, R.T. 1965. Experimental rabies: Studies of cellular vulnerability and pathogenesis using fluorescent antibody staining. J. Neuropathol. Exp. Neurol. 24:662-674.

Fixed rabies virus infections of mice were studied with fluorescent antibody staining in addition to conventional histologic and virologic methods. Following intracerebral or subcutaneous inoculation, virus was detectable only in neurons, and infection was limited primarily to neurons of rhinencephalic structures, brain stem nuclei, dorsal root ganglia, anterior horns, and cerebellar Purkinje cells. During the incubation period of infection, antigen developed slowly over 5 days within the perikaryon and dendrites of susceptible neurons, and no antigen was found in axons. Following subcutaneous inoculation virus spread rapidly to the CNS via peripheral nerves without evidence of infection of the endoneurial cells or any extraneural tissues. These findings are different from those observed with other experimental viral encephalitis.

5744

Johnson, R.T.; Mercer, E.H. 1964. The development of fixed rabies virus in mouse brain. *Australian J. Exp. Biol. Med. Sci.* 42:449-456.

The development of fixed rabies virus in the cerebellar Purkinje cells of mice has been studied by immunofluorescent, cytochemical, and electron microscopical methods. The virus, as judged by all methods, appears to develop in the cytoplasm without any demonstrable nuclear involvement. The staining reaction with acridine orange suggests that rabies is an RNA virus. The morphology of the particles of the fixed strain used in these experiments differs from the street strain in being less often elongated and unbranched.

5745

Kissling, R.E.; Reese, D.R. 1963. Anti-rabies vaccine of tissue culture origin. *J. Immunol.* 91:362-368.

Formalin-inactivated vaccines of sufficient potency to satisfy the NIH standards have been prepared by concentration of rabies virus grown in hamster kidney tissue cultures. The cytopathic effect produced by tissue culture - adapted rabies virus has permitted the performance of in vitro neutralization tests. Rabies virus possesses essential lipid and is probably a ribonucleic acid type of virus.

5746

Larsh, S.E. 1965. Indirect fluorescent antibody and serum neutralization response in pre-exposure prophylaxis against rabies. *Ann. Intern. Med.* 63:955-964.

A shortened time schedule for pre-exposure immunization against rabies has been evaluated by the indirect fluorescent rabies antibody test and the standard serum neutralization test. By the former, all subjects tested were positive; by the latter, 84 per cent were positive. Comparison of this study on the indirect fluorescent rabies antibody test and serum neutralization response with a large study at the Communicable Disease Center revealed the great accuracy of the indirect fluorescent rabies antibody test, with no false positives. The advantages of that test over the serum neutralization test are its greater simplicity, sensitivity, and rapidity of performance. Moreover, since results are quickly available, the need for additional boosters can be determined immediately. Studies are currently underway to attempt to evaluate the significance of the indirect fluorescent rabies antibody-serum neutralization titer elevation in relation to protection against rabies challenge. The direct fluorescent antibody test has been used more widely than the indirect, and has replaced routine animal inocu-

lation in the diagnosis of rabies in some states. Indications for rabies pre-exposure immunization can best be evaluated when certain factors of prevalence and pathogenesis in animals are considered.

5747

Leffingwell, L.; Irons, J.V. 1965. Rabies antibodies in human sera titrated by the indirect FA method. Public Health Rep. 80:999-1004.

The indirect fluorescent rabies antibody (IFRA) test was evaluated for its usefulness in titrating rabies serum antibodies. The titers obtained were compared with the logs of virus protected in the serum neutralization (SN) test. There was complete agreement between the two tests for 93 per cent of the sera. In all instances where there were several sera from one person, the indirect fluorescent antibody method detected the increases in titer, and in three instances the FA response was noted first. This initial response was subsequently confirmed in later specimens by rises in titer by both the IFRA and SN methods. In no instance did the SN test detect the initial response before the IFRA test. These results suggest that the indirect fluorescent antibody method may be the more sensitive. If so, it could ultimately supplant the neutralization test as a sensitive, rapid, high-resolution diagnostic method in comparing titers of pre-vaccine and post-vaccine sera.

5748

Lennette, E.H.; Woodie, J.D.; Nakamura, K.; Magoffin, R.L. 1965. The diagnosis of rabies by fluorescent antibody method employing immune hamster serum. Health Lab. Sci. 2:24-34.

Antirabies immune sera were prepared by the inoculation of hamsters with three or four serial doses of beta-propiolactone - inactivated suspension of CVS strain of fixed rabies virus in Arlacel-mineral oil adjuvant followed by two doses of live virus suspension. Fluorescein-conjugated immune globulins prepared from these hamster sera were consistently superior in staining quality to the immune horse serum conjugates previously employed in fluorescent antibody tests for rabies. An evaluation of the FA test, employing hamster immune serum conjugates, included examination of approximately 4,200 specimens. About three-fourths of the specimens were shipped in glycerine-saline preservative; the remainder were fresh or frozen. An array of species was represented, with dogs, cats, skunks, and bats being the most frequent. From the combined results of FA tests, examinations for Negri bodies, and the inoculation of mice, 363 of the approximately 4,200 specimens were positive for rabies; 99.4 per cent were detected by the FA test, 98.3 per cent by inoculation of mice, and 65.8 per cent by the presence of Negri bodies. When fluorescein-globulin conjugates of good staining quality are employed, FA is as sensitive as mouse inoculation.

5749

Lipkin, M.E.; Kishko, Ya.G.; Urin, A.I.; Kolotilova, L.V.; Ionov, L.I. 1965. Use of the fluorescence method for revealing poliomyelitis and rabies viruses. Vop. Virusol. 10:26-29. In Russian.

Possibilities were investigated for identifying poliomyelitis and ECHO-6 viruses at various periods after infection of HeLa cells by fluorescein-labeled anticomplementary serum. Four hours after inoculation of viruses specific changes were detected in the cytoplasm. Then inclusions appeared, the number of which sharply increased by 24 hours. Poliomyelitis virus could be differentiated from ECHO-6 virus. By using fluorescein-stained human antiserum, specific inclusions were disclosed in smears from Ammons horn of a man who died of rabies.

5750

Nadel, M.K.; Orsi, E.V. 1964. Immunofluorescence of non-cytopathic tissue-culture-adapted fixed rabies. Amer. J. Med. Technol. 30:173-176.

The fixed strain of rabies virus adapted to chick embryonic monolayers fails to elicit pathologic changes in the infected host cell when viewed by conventional light microscopy. Immunofluorescent microscopic examination of such infected cells indicates the presence of specific intracytoplasmic viral inclusions.

5751

Otani, S. 1965. Studies on rabies encephalitis by means of fluorescent antibody staining. Jap. J. Exp. Med. 35:42P-44P.

Direct FA was used to follow the histologic course of rabies infection in mice and hamsters. Spread of virus was correlated with time. Many nervous tissues were eventually involved. It is still not clear how the virus is spread within the body. Salivary gland infection did not seem to play a role in viremia production.

5752

Phillips, M.S.; Bratton, J.K.; Janes, R.L.; Masterson, R.A. 1963. Fourteen-day clinical course of rabies in a cow. J. Amer. Vet. Med. Ass. 143:1216.

FA was used to diagnose this case.

5753

Schaap, G.J.P.; Velthoen, A.A. 1962. Technical remarks on the application of the fluorescent antibody test (FA test) in the diagnosis of rabies. *Antonie van Leeuwenhoek J. Microbiol. Serol.* 28:216.

Rabies virus was detected in impression smears of animal brains. FITC-labeled purified globulin preparations gave as good results as labeled whole serum. Nonspecific staining of brain tissue was prevented by absorption of conjugates with acetone-dried mouse brain powder, but nonspecific fluorescence of granules of eosinophilic leukocytes could not be prevented. The indirect FA test is useful for rapid detection of antibody formation in persons being vaccinated against rabies.

5754

Sokolov, N.N.; Parfanovich, M.I. 1955. Accumulation of specific antigen and distribution of nucleic acids in sheep embryo kidney cells infected with street rabies virus as revealed by fluorescence microscopy. *Acta Virol.* 9:191.

Staining of the preparations with anti-rabic gamma globulin labeled with fluorescein isothiocyanate revealed specific rabies virus antigen in the nucleoli and on the nuclear membrane starting from 24 hours after inoculation. After 48 hours the antigen was demonstrated in the nuclei as granular agglomerates and in cytoplasmic inclusions; after 5 days, in addition to this, homogeneous specific fluorescence of the whole cytoplasm was observed in the majority of cells.

5755

Thomas, J.B.; Sikes, R.K.; Ricker, A.S. 1963. Evaluation of indirect fluorescent antibody technique for detection of rabies antibody in human sera. *J. Immunol.* 91:721-723.

Five hundred and eighty-eight sera were tested by the serum neutralization (SN) and indirect fluorescent rabies antibody (IFRA) procedures. Of 285 positive sera by the SN test, 98.3 per cent were also positive by the IFRA method, indicating acceptability of the IFRA test as a screening procedure for the detection of rabies antibody. Seventy-eight sera from rabies-immunized people were positive by the IFRA method and negative by the SN method, indicating the greater sensitivity of the IFRA test. Further investigations are needed to define the relationship of demonstrable rabies antibody to protection against rabies virus challenge.

5756

Wiktor, T.J.; Fernandes, M.V.; Koprowski, H. 1964. Cultivation of rabies virus in human diploid cell strain WI-38. *J. Immunol.* 93:353-366.

In spite of the initial infectivity of three strains of rabies virus for the human diploid cell strain WI-38, the virus could not be serially propagated in this cell system with conventional methods of passaging infectious material. However, by following the mixing-cell transfer technique, three strains could finally be adapted so that they could propagate indefinitely. The adapted HEP virus caused a cytopathic effect in WI-38. Its infectivity for four other tissue culture systems of different animal origins increased during the course of its propagation in WI-38. After adaptation to WI-38, the HEP strain seemed to lose its lethal properties for monkeys injected intracerebrally, but acquired a high degree of immunizing capacity for the same species. Inactivated vaccines prepared from two other strains of fixed virus, CVS and PM, were highly antigenic after adaptation to WI-38. This fact and the availability of the WI-38-adapted live HEP virus seem to indicate that production of effective and safe anti-rabies vaccine for man is now feasible.

5757

Wiktor, T.J.; Fernandes, M.V.; Koprowski, H. 1965. Detection of a lymphocytic choriomeningitis component in rabies virus preparations. *J. Bacteriol.* 90:1494-1495.

Tissue cultures chronically infected with rabies virus were examined by direct and indirect FA. Lymphocytic choriomeningitis virus was found in 9 of 11 rabies-infected tissue cultures. Similar tissue cultures, either noninfected or infected with other viruses, did not yield lymphocytic choriomeningitis virus. This is thought not to be a casual relationship.

5758

Yamamoto, T.; Otani, S.; Shiraki, H. 1965. A study of the evolution of viral infection in experimental herpes simplex encephalitis and rabies by means of fluorescent antibody. *Acta Neuropathol.* 5:288-306.

The routes of access of herpes simplex virus into the suckling mouse central nervous system are hematogenous dissemination that affects mainly the cerebrum and cerebellum, and a neural pathway by the spinal cord and the autonomic system. The main hosts in herpetic infection of the nervous systems are neurones, astrocytes, and Schwann cells. Most of the viral antigen is confined to the cytoplasm. The most common visceral sites for herpes virus multiplication are the intestine and

lung. The host in rabies infection is the nerve cell. Virus antigen is distributed in both peripheral processes and cytoplasm. In the ganglia of both cranial and spinal nerves, brain stem, and diencephalon, rabies virus antigen appears at an earlier stage of infection and is larger in amount than in the cerebrum and cerebellum. Fine particles of rabies virus antigen in acinar cells of the salivary gland first appear 3 days after infection, gradually aggregate in the cellular periphery around the acinar space, and finally agglomerate into coarse granules located in the acinar lumina 9 days after inoculation.

VII. PICORNAVIRUS

5759

Barquet, J.S.; Alvarez, B.; Epstein, B. 1964. Susceptibility of cells to the T9 strain of poliomyelitis viruses I, II, and III as studied by aminoacridine and fluorescent antibody stains. *Medicina* 24:98-101. In Spanish.

A study was performed on the lesions provoked by the three types of poliomyelitic virus on the T9 strain through direct observation, staining with fluorescent substances, immunofluorescence, and a neutralization test, showing in this way the susceptibility of HeLa cells to polio virus.

5760

Bosella, F. 1964. A comparative demonstration of antibodies against poliomyelitis virus using the neutralization and immunofluorescence tests. *Boll. Ist. Sieroterap. Milan* 43:249-252. In Italian.

Levels of polio antibodies have been comparatively determined in the sera of 13 subjects before and after vaccination against poliomyelitis (live Sabin vaccine) by the neutralization technique and the indirect immunofluorescence technique. Both methods gave comparable results. However, antibody levels detected by means of immunofluorescence were usually lower than by the neutralization technique.

5761

Butler, M.; Leach, R.H. 1964. A mycoplasma which induces acidity and cytopathic effect in tissue culture. *J. Gen. Microbiol.* 34:285-294.

An agent that induced acidity and cytopathic effects in Hep-2 tissue cultures was investigated. The agent grew well in certain other tissue culture systems. Typical mycoplasma colonies were isolated from the contaminated Hep-2 cultures and on reinoculation into Hep-2 cultures produced effects indistinguishable from the original effects. There was no appreciable growth in tissue culture medium alone. The mycoplasma had biological properties similar to those of known mycoplasmas, including Mycoplasma hominis Type 1, but was serologically distinct from these. Fluorescent antibody and Giemsa-staining techniques showed extracellular forms. Other mycoplasmas were shown to grow in tissue culture; M. gallisepticum induced similar effects to the cytopathic agent but was distinct in serological and biological properties. The agent partially inhibited the growth of measles virus.

5762

Clark, H.F.; Shepard, C.C. 1963. A dialysis technique for preparing fluorescent antibody. *Virology* 20:642-644.

A dialysis method is described for conjugating serum proteins with FITC. Antisera against rabies, polio, rickettsiae, and mycobacteria were used. Human, monkey, guinea pig, sheep, and rabbit globulins were conjugated by this method. Good specific FA staining with minimal nonspecific staining was obtained.

5763

Crocker, T.T.; Pfendt, E.; Spendlove, R. 1964. Poliovirus: Growth in non-nucleate cytoplasm. *Science* 145:401-403.

Cytoplasmic fragments were produced by micromanipulation of cells from a human amnion cell line cultured on cover slips. The cultures were infected with Type 1 (Mahoney) poliovirus, and incubated for 7 hours with tritiated uridine (H3U). FA to the poliovirus indicated antigenic sites in a number of non-nucleate fragments. By autoradiography the incorporation of H3U was demonstrated at some of the same sites. The occurrence of poliovirus antigen at the same site as induced synthesis of RNA in non-nucleate cytoplasm of mammalian cells indicates that poliovirus infection and growth occurred independently of immediate contribution from the nucleus.

5764

Crocker, T.T.; Pfendt, E.; Spendlove, R. 1964. Growth of poliovirus in non-nucleate cytoplasm. *Federation Proc.* 23:868:245.

Dependence of RNA viruses on nuclear mechanisms for reproduction has been rendered unlikely but not disproved by observations in actinomycin-treated cells and by synthesis of some viral components in cell-free systems. Non-nucleate cytoplasmic fragments were produced by micromanipulation in cover slip cultures of a human amnion line. Cultures were then infected with poliovirus, Type I, Mahoney, and incubated for 7 hours with uridine labeled with tritium. Fluorescent antipolio antibody demonstrated antigenic sites in a number of non-nucleate fragments as well as in most intact cells. Autoradiography demonstrated labeled uridine incorporation at the same sites. Occurrence of poliovirus-induced RNA synthesis and poliovirus antigen at the same site in non-nucleate cytoplasm indicates that poliovirus infection and growth occurred independently of nuclear control or participation. Complete article.

5765

Frommham, L.H.; Martins, M.J. 1963. A comparison of fluorescein-labeled gamma globulins purified by Rivanol and DEAE chromatography. J. Immunol. 90:116-120.

Preparations of fluorescein-labeled gamma globulins obtained by the Rivanol procedure were found to be comparable to DEAE gamma globulins in recovery of antibody and specificity of fluorescent staining. These preparations were applied to the fluorescent antibody analysis of the V antigens of influenza virus, where it was necessary to use purified, labeled gamma globulins to achieve full specificity. However, in other virus-cell systems immune globulins prepared by ammonium sulfate precipitation, conjugated at low ratios of fluorescein to protein, and passed through a Sephadex column were found to be comparable to the Rivanol and DEAE globulins in specificity and, at times, superior in stainability. In the reverse situation, where essentially no contaminating host materials are present, as with preparations of bacteria, the use of ammonium sulfate globulins conjugated at high F:P ratios seems to afford the greatest intensity of staining.

5766

Godman, G.C.; Rifkind, R.A.; Page, R.B.; Howe, C.; Rose, H.M. 1964. A description of ECHO-9 virus infection in cultured cells: II. Cytochemical observations. Amer. J. Pathol. 44:215-245.

At about 4 hours after ECHO-9 infection, perinuclear accumulation of ribonucleoprotein is followed by loss of nonhistone protein and RNA from the nucleus, flocculation of chromatin, and collapse of the nucleus. Nuclear function (RNA synthesis) stops, but at a stage early in infection apparent primary cytoplasmic incorporation into RNA of H3-uridine occurs. Thrombonucleoprotein of the cytoplasm, grossly unchanged in amount, is gathered into masses, consisting of linear clusters of ribosomes, perinuclearly and at the cell periphery. Antigen, determined by FA, is accumulated and evidently synthesized in these clusters. Viral particles, however, are assembled at definite sites spatially distinct from the presumed ribosomal sites of synthesis of viral components. Other structures and functions are discussed.

5767

Hannoun, C.; Fernandes, M.V.; Macleira-Coelho, A. 1965. Effects of cortisone on in vitro viral infection: I. Inhibition of cytopathogenesis by polio, rabies, and yellow fever viruses in human embryonic cells. *Proc. Soc. Exp. Biol. Med.* 119:153-158.

Plaque formation, observation of cytopathic effect, immunofluorescence, and mouse inoculation were used variously to evaluate some aspects of cortisone acetate on infection of diploid human embryonic fibroblastic cultures with polio, rabies, and yellow fever viruses. Cortisone did not affect poliovirus directly, but did inhibit plaque production when cells were subjected to many passages in its presence. Both accumulation of cytopathic effect and spread of virus in cultures infected with rabies virus before serial subculture were inhibited by cortisone in passage medium. Cortisone exerted a similar cumulative effect on virus production by cells infected with yellow fever virus, and an immediate inhibition of cytopathic response, but both virus production and cytopathic effect returned on removal of cortisone from the medium. The findings suggested a cumulative but nonselective effect of cortisone on cellular capacity to respond to virus infection.

5768

Hatch, M.H. 1963. Identification of Coxsackie and ECHO virus isolates with fluorescent antibody. *Proc. Soc. Exp. Biol. Med.* 114:161-165.

A direct fluorescent antibody staining method is reported for laboratory identification of three Coxsackie and three ECHO viruses isolated from stool specimens. Monkey kidney tissue cultures inoculated with stool suspensions are stained when they show early cytopathic effect. Seventy stools were studied as unknowns by the fluorescent antibody method; 55 of these had previously been found to contain one of the six viruses by routine procedures involving neutralization tests. Fifty-one were correctly identified by the fluorescent antibody method. Of the four failures, three were found negative and one was incorrectly typed. Fifteen specimens shown not to contain any of the six viruses by the routine method were negative by fluorescent antibody. The reasons for the four failures with fluorescent antibody were investigated and modifications introduced into the method that should make it as sensitive and specific as the neutralization tests. The viruses were identified much more rapidly with fluorescent antibody.

5769

Henle, G.; Henle, W. 1965. Cross-reactions among gamma globulins of various species in indirect immunofluorescence. *J. Immunol.* 95:118-124.

Several preparations of fluorescein isothiocyanate - conjugated rabbit antibodies to guinea pig or human gamma globulin fractions were found to be capable of staining the characteristic mumps virus inclusions in infected HeLa cells previously sensitized with virus-specific antibodies derived from heterologous animal species. Brilliant immunofluorescence was elicited by labeled antibodies to heterologous gamma globulins only when the antiviral sera used for the primary stage of the reaction were used undiluted, or at the most in 10-fold dilution. The rabbit anti-guinea pig gamma globulin sera from which effective fluorescein conjugates were prepared yielded from one to three lines of precipitation in Ouchterlony tests with sera from several heterologous species with the exception of rabbits, whereas those sera from which strictly species-specific conjugates were obtained produced no precipitates with heterologous sera. Cross-reacting antisera to guinea pig gamma globulin also reduced the viral neutralizing capacity of human poliomyelitis convalescent serum. No attempts were made to identify the cross-reacting serum components. Such broadly reactive anti-gamma-globulins might be useful for the indirect technique of immunofluorescence in that they overcome the usually observed species limitation of this method.

5770

Ishida, N. 1963. Analysis of the nature of infectious virus disease by the fluorescent antibody method. *Nippon Densenbyo Gakkai Zasshi* 37:212-218. In Japanese.

The progress of influenza virus PR8 infection in the mouse was followed at the cellular level by the fluorescent antibody method. Virus accumulated in the kidney as well as in the pulmonary epithelium. The diagnosis of fluorescent decidual cells was also applied to Coxsackie B5 and measles infections. Fluorescent antibody is useful in locating the site of contagious virus infections.

5771

Jamison, R.M.; Mayor, H.D.; Melnick, J.L. 1963. Studies on ECHO 4 virus (picornavirus group) and its intracellular development. *Exp. Mol. Pathol.* 21:188-202.

A detailed study has been made of two strains of ECHO 4 virus, one of which is not readily neutralized even with homologous antisera. The intracellular development of the prototype Pesascek strain and of the DuToit strain was followed in monkey kidney cultures. Cells infected with either strain began to produce viral antigen at approximately 3

hours after inoculation, as measured by immunofluorescence. The time course and sequence of viral antigen formation was identical in cells infected with each strain. Cytoplasmic RNA increased 2 hours earlier in cells infected with the DuToit strain over those infected with the Pesascek. Other characteristics of the virus are described.

5772

Kokles, R.; Urbaneck, D. 1964. Immunohistologic and cytopathologic results of infection with foot-and-mouth disease virus on cell cultures from calf kidneys. Arch. Exp. Veterinaarmed. 18:6:1471-1481. In German.

Cell cultures from calf kidneys were infected with foot-and-mouth disease virus and subjected to FA tests. Several phases of cell fluorescence were observed that differed as to localization and intensity, but agreed with the degree of the cytopathologic changes. BA-46-67257.

5773

Korych, B.; Chyle, M.; Franek, J. 1965. The kinetics of multiplication of Teschen disease virus (TDV) in an established porcine kidney cell line (strain PK): Correlation between cell response and virus release. J. Hyg. Epidemiol. Microbiol. Immunol. 9:31-36.

In our initial experiments with the cultivation of TDV in primary cell cultures of embryo pig kidney, two stages of cytopathic changes could be distinguished morphologically in native preparations. Specific granulations, demonstrable by FA, were compared with the development of morphological reactions using phase contrast. Specific fluorescence was apparent 4 hours after infection. In the following hour fluorescence increased in quantity and intensity. Later, a gradual diminution was observed. After a lapse of another 4 hours there was a new increase. Granular fluorescence was found in the cytoplasm only, chiefly in the proximity of nuclear membranes. Fluorescence in the nucleus was detected only in cases of nuclear destruction accompanied by chromatolysis. FA and phase contrast microscopy suggest that changes appear to come in approximately 4 hour cycles. New extracellular virus in the medium of infected cell cultures prepared for FA appeared 8 hours after infection. Cytopathic changes in cells coincided with virus release.

5774

Kovacs, E.; Baratawidjaja, R.K.; Labzoffsky, N.A. 1963. Visualization of poliovirus Type III in paraffin sections of monkey spinal cord by indirect immunofluorescence. Nature 200:497-498.

The presence of viral antigen with unmistakable fluorescence was observed mainly in motor neurones, inflammatory cells, and vessel walls or perivascular cuffings. Virus antigen in round cells or macrophages is shown in the area of neuronophagic lesion. This fluorescence is assumed to be due to the presence of the viral antigen, since the

same picture was not observed in the control preparations. The similar picture, as described and illustrated here, was observed in the sections of the spinal cord of another monkey infected with poliovirus Type III.

5775

Kovacs, E.; Baratawidjaja, R.K.; Walmsley-Hewson, A.; Labzoffsky, N.A. 1963. Demonstration of poliovirus in isolated leucocytes, blood and C.S.F. smears of infected monkey by immunofluorescence and biological means. Arch. Ges. Virusforsch. 14:141-146.

Poliovirus was detected by FA in leukocytes of blood and cerebrospinal fluid smears. This may be useful for diagnosis. Results with influenza, lymphocytic choriomeningitis, mumps, and rabies were also encouraging.

5776

Larson, V.M. 1964. A study of Coxsackie A14 virus in experimental animals (rodents) and in tissue culture by means of immunofluorescence. Diss. Abstr. 25:2458.

Adaptation of two strains of Coxsackie A14 virus was attempted by serial passage in several tissue culture systems. The prototype Coxsackie A14 virus was propagated in primary human amnion cells and in a continuous monkey kidney cell line, LLC-MK2, but not in a continuous human amnion cell line. The adult mouse adapted strain of Coxsackie A14 did not produce TPE. Coxsackie A14 antigen and morphological cellular changes were studied at intervals during the virus growth cycle in primary human amnion and LLC-MK2 cells by electron microscopy and by Giemsa, acridine orange, and direct FA stains. In the study utilizing LLC-MK2 cells, free and cell-associated infective virus was also determined by titration of supernatant fluid and of cells after rapid freezing and thawing. The sequential development of viral antigen in primary human amnion and LLC-MK2 cells was similar. Viral antigen was first detected in the perinuclear region at 6 hours. By 12 hours, viral antigen characteristically assumed a prominent punctate distribution throughout the cytoplasm. Later the cells developed cytopathic rounding but continued to retain large quantities of viral antigen. The production and release of infective virus in LLC-MK2 cultures correlated with the appearance of viral antigen. The susceptibility of mice and Mongolian gerbils to the adult mouse adapted strain of Coxsackie A14 was studied in relation to route of inoculation and age of the host. Tissues from adult gerbils inoculated subcutaneously were studied by immunofluorescence and infectivity titration. The paralytic disease induced in these animals was found to be of neural origin.

5777

Lipkin, M.E.; Kishko, Ya.G.; Urin, A.I.; Kolotilova, L.V.; Ionov, L.I. 1965. Use of the fluorescence method for revealing poliomyelitis and rabies viruses. Vop. Virusol. 10:26-29. In Russian.

Possibilities were investigated for identifying poliomyelitis and ECHO-6 viruses at various periods after infection of HeLa cells by fluorescein-labeled anticomplementary serum. Four hours after inoculation of viruses specific changes were detected in the cytoplasm. Then inclusions appeared, the number of which sharply increased by 24 hours. Poliomyelitis virus could be differentiated from ECHO-6 virus. By using fluorescein-stained human antiserum, specific inclusions were disclosed in smears from Ammons horn of a man who died of rabies.

5778

Liu, C.; Sharp, M.E. 1964. Immunofluorescent studies of pathogenesis of encephalomyelitis in mice. Federation Proc. 23:555:193.

Theilers GD VII virus was inoculated to mice intracerebrally, intranasally, and by feeding. Encephalitis developed 4 to 5 days after intracerebral inoculation, with infectious virus in the brain attaining 1 million doses lethal for 50 per cent. Viral antigen was detected in neurons by both direct and indirect fluorescent antibody staining in areas of the thalamus, brain stem, and spinal cord. The spread of virus appeared to follow the course of the hippocampus. Following intranasal inoculation the GD VII virus entered the central nervous system through the olfactory bulb and spread to the brain stem via the hippocampus. The incubation period was 7 to 8 days. By feeding the virus with polyethylene gavage tube, mice developed encephalitis 6 to 14 days after feeding, with a mean of 9.1 days. Moderate amount of infectious virus was found in the heart, lung, and intestines 1 day after feeding and only a small amount of virus was detectable thereafter. When encephalitis developed, 4 to 5 logs of infectious virus were recovered from the brain and spinal cord. By immunofluorescent staining, no viral antigen was detectable in any of the visceral organs such as lung, spleen, liver, intestines, and kidney. Viral antigen was observed in the neurons of the central nervous system when encephalitis was apparent. Complete article.

5779

Olenchnowitz, A.-F.; Kokles, R.; Urbaneck, D. 1965. Experiences in the preparation of purified fluorescent antibodies. Arch. Exp. Veterinaarmed. 19:139-148. In German.

Globulins from bovine foot-and-mouth disease hyperimmune serum are precipitated with ammonium sulfate and coupled with FITC. The conjugates are purified by gel filtration and chromatography. The dye-protein ratio largely determines the staining properties of the conjugates. The importance of their extent of coupling and antibody content for the specificity of antibody determination is discussed and optimal conditions for preparing conjugates without unspecific fluorescence are postulated. BA-46-80842.

5780

Philipson, L.; Dinter, Z. 1963. The role of interferon in persistent infection with foot-and-mouth disease virus. J. Gen. Microbiol. 32:277-285.

Calf kidney cell cultures persistently infected with foot-and-mouth disease virus, resist challenge with related and unrelated viruses. Attachment of challenge virus to persistently infected cells is not impaired. A challenge with viral ribonucleic acid will not overcome the resistance of persistently infected cells. The initiation of persistence is correlated with the amount of interferon produced in the cells. It is concluded that interferon plays a major role in initiation and maintenance of the carrier state of the cells. Indirect FA was used.

5781

Rabin, E.R.; Hassan, S.A.; Jenson, A.B.; Melnick, J.L. 1964. Coxsackie virus B3 myocarditis in mice: An electron microscopic, immuno-fluorescent, and virus assay study. Amer. J. Pathol. 44:775-779.

White Swiss mice between 12 and 20 days of age frequently developed severe myocarditis after inoculation with Coxsackie B3 virus. Myocardial damage was slightly more extensive in animals receiving virus plus cortisone than in animals receiving virus alone, but by the 7th post-inoculation day damage was usually so severe in both groups that lesions were apparent on gross inspection of the heart. Virus was recovered in high titers in the heart during the period when myocardial injury occurred and after viremia was no longer detectable. Viral antigen, demonstrated by the direct fluorescent antibody method, was present in damaged myocardial fibers and intact fibers surrounding foci of necrosis. Specific staining was often intense around muscle cell nuclei.

5782

Rivenson, S.; Planes de Banchemo, E.; Zakin, M.M. 1962. Identification of the aphthous virus using the fluorescent antibody technique. *Rev. Invest. Ganaderas* 15:275-284.

Using FA, it was possible to see the antigen-antibody reaction between the three types of foot-and-mouth disease virus (O, A, and C) and their homologous sera. Smears of bovine tongue epithelium and monolayers of pig kidney cells were used. FA was not a satisfactory method for the detection of specific antibody in the sera of cattle convalescent from the disease. FITC was more satisfactory than RB 200 as a labeling agent. The superiority of the gamma globulins of the rabbit sera to total serum and complete globulins was demonstrated. The gamma globulins were fractionated by precipitation with ammonium sulfate 50 per cent saturation and by passage on DEAE cellulose, without notable differences. The labeled gamma globulins were purified by passage on DEAE cellulose and by adsorption on tissue powder. With the first method nonspecific fluorescence was eliminated.

5783

Sommerville, R.G.; MacFarlane, P. 1964. The rapid diagnosis of virus infections by immunofluorescent techniques applied to blood leukocytes. *Lancet* 1:911-912.

The application of the fluorescent antibody technique to peripheral blood leukocytes offers a new method for the rapid diagnosis of virus infections.

5784

Tobe, T. 1965. Inapparent virus infection as a trigger of appendicitis. *Lancet* 1:1343-1346.

During the past 5 years 642 appendices were removed and examined microscopically. Lymphoid hyperplasia in various degrees was found in 91 per cent of the 474 infected cases. Many sera of patients with appendicitis showed raised complement fixation titers against Coxsackie-B virus or adenovirus. With a fluorescent antibody technique, Coxsackie-B virus and adenovirus were clearly demonstrated in the cytoplasm of histiocytes adjacent to the epithelium of the mucous membrane of the appendix, in the cytoplasm of reticulum cells in the lymph follicles of the appendix, and in the cytoplasm of hyperplastic mesenteric lymph nodes in many cases. Tissue cultures have so far been negative. Inapparent virus infection plays an important part as a trigger of appendicitis.

5785

Urbaneck, D.; Kokles, R.; Olenchnowitz, A.-F. 1964. Immunological localization studies of a neurotropic foot-and-mouth disease virus in the brains of white mice. Arch. Exp. Veterinaarmed. 18:1203-1216. In German.

White mice were infected with neurotropic foot-and-mouth disease virus by intracerebral and intraperitoneal injection respectively. Their brain were examined by means of fluorescent antibodies. The virus was regularly found in the ganglial cells and in the ependymal cells of the plexus chorioidei of the lateral ventricles. Mesenchymal borders of the brain are rarely affected. BA-46-39756.

5786

Vandeputte, M. 1964. Application of the immunofluorescence technique in virology and anatomic pathology. Acta Clin. Belg. 19:217-218. In Flemish.

The applications of the immunofluorescence technique in experimental pathology and virology are widespread. This technique was applied in the search for the viral antigen in tumors of the rat caused by the SE poliomyelitis virus and also in the tissue cultures of these tumors. With this technique the author demonstrated that although no viral antigen is present in the tumors themselves, this antigen is synthesized by the cancer cells in a certain percentage of test tube cultures of these growths. This technique also enabled him to follow the synthesis of the viral antigen morphologically on the cytologic level. A number of technical problems still have to be solved before this method becomes routine, among them, autofluorescence and non-specific fluorescence.

5787

Voronina, F.V.; Pille, E.R. 1963. A study of multiplication of simian viruses in tissue culture by fluorescent antibody method. Vop. Virusol. 8:594-600. In Russian.

The distribution in cultured monkey kidney cells of three simian viruses of different groups was studied by immunofluorescence. The accumulation of antigen in cells was compared with the development of the cytopathic effect in acridine orange stained preparations and with the accumulation of virus in the cultural fluid. The antigen of the virus of the first groups, which is similar to adenoviruses, accumulated in the nucleus in DNA-containing grains and dense inclusion bodies; at later stages the virus antigen appeared in the cytoplasm. Antigens of the viruses of the second and third groups, which are similar to enteroviruses,

are localized only in the cytoplasm. A parallelism is noted between the accumulation of antigen in cells and the increase of virus titer in the cultural fluid. Differences in the distribution and time of appearance of antigens of the three virus groups are described.

5788

Zalan, E.; Kelen, A.E.; Labzoffsky, N.A. 1965. Immunofluorescence studies on Cocksackie Group A viruses: I. Localization of viral antigen in infected primary human amnion cells. Arch. Ges. Virusforsch. 15:668-680.

Indirect FA was found suitable for the detection and specific identification of viral antigens in primary human amnion cells infected with tissue culture adapted Cocksackie Group A viruses. The process of adaptation as well as sequential changes in the development of viral antigen were observed with the aid of FA. Some cross-reactions were noted between some of the serotypes. These, however, could easily be differentiated from type-specific reactions by the intensity of fluorescence. Possibilities of the practical application of FA in the laboratory diagnosis of Cocksackie A virus infections are discussed.

VIII. MYXOVIRUS (not influenza)

5789

Atherton, J.G.; Chaparas, S.D.; Cremer, M.; Gordon, I. 1965. Mechanism of polykaryocytosis associated with noncytopathic infection by measles virus. J. Bacteriol. 90:213-219.

Infection with a measles virus variant resulted not only in formation of polykaryocytes (PK) but also in formation of multicellular immunofluorescent foci (IFF) in which no cytopathic effect could be detected. The ratio of IFF to PK changed from 27 to 4 during the first passage and remained 4 after a second passage. PK were plaques. Plaque assay was linear in the presence of IFF. To investigate the mechanism of PK formation, radioautography was done on cells pulse-labeled with tritiated thymidine before virus multiplication began. The results showed that PK were formed by fusion; there were no PK whose nuclei contained no label, and the proportion of labeled nuclei (32 per cent) and the distribution of grain counts was the same in PK as in uninvolved cells, ruling out nuclear replication without concomitant cytoplasmic membrane formation as the mechanism of formation of these PK. Early in PK development, neutral red uptake was markedly increased, causing red plaques. As PK matured, hyperchromicity disappeared, resulting in white plaques. This sequence provided an index of rate of evolution of PK. Rate of PK maturation was more rapid at 37 than at 32 C.

5790

Azadova, N.B.; Uryvayev, L.V.; Zhdanov, V.M. 1965. Synthesis of components of Sendai virus in the presence of proflavine. J. Immunol. 95:791-793.

Proflavine sharply alters the processes of transport of S antigen of Sendai virus from the nucleolus to the cytoplasm, but does not essentially decrease its synthesis. The decrease in synthesis of the V antigen is proportionately more marked. Retention of S antigen in the nucleolus leads to the formation of incomplete virus and to a reduction of release of infectious virus from the cell. Interaction of proflavine with the viral RNA does not essentially alter the messenger mechanism responsible for synthesis of protein components of the virus.

5791

Bals, M.; Copelovici, Y.; Stratti, I.; Strulovici, D. 1964. Cultivation of the mumps virus in chick embryo cell cultures, by immunofluorescence. *Rev. Roumaine Inframicrobiol.* 1:103-108.

By means of FA, the mumps antigen was made evident in chick embryo cell cultures infected with an adapted mumps virus strain, within 3 or 5 hours, depending upon the virus dose inoculated. Visualization of the antigen by FA preceded infectivity and hemagglutination.

5792

Baublis, J.V.; Brown, G.C. 1965. Rubella antibodies in fractions of cord and convalescent serum. *Federation Proc.* 24:667:249.

Convalescent serum from clinical cases of rubella and cord serum from infants whose mothers had the disease during pregnancy were fractionated by Sephadex G-200 filtration, ammonium sulfate precipitation, and starch block electrophoresis. In addition, convalescent serum was fractionated by cold ethanol precipitation, Cohn Method 6-9. Various fractions were tested for rubella antibodies by the indirect method of immunofluorescence and by neutralization techniques in tissue culture. 19S globulin from cord blood and early convalescence was positive, but not later. 7S fractions were positive whenever whole serum contained antibodies. Anti-rubella activity was demonstrated in Cohn Fractions II-III, II 1-2, and III obtained by Method 6-9, in globulin precipitated with ammonium sulfate, and in the more rapid gamma zone by starch block electrophoresis. Complete article.

5793

Blaskovic, D.; Szanto, J.; Albrecht, P.; Sadecky, E.; Lackovic, V. 1964. Demonstration of swine influenza virus in pigs by the fluorescent antibody method. *Acta Virol.* 8:401-409.

An early diagnosis of influenza in pigs was attempted. Eight-week-old pigs were inoculated intranasally with a massive dose of swine influenza A virus strain Iowa 15. Clinical symptoms developed in all the animals from 2 days after inoculation. The presence of the virus in various tissues and organs of the respiratory tract throughout the observation period, lasting from the 1st to the 5th day after inoculation, was established by isolation experiments. Viral antigen was revealed by the fluorescent antibody technique from the 3rd day after infection in sections from the turbinates and in the lungs, almost exclusively in the peripheral parts of pneumonic areas (endemic or virus pneumonia of pigs) and in the adjoining aerated lung tissue. Distinct nuclear or diffuse fluorescence were characteristic of early or advanced infection of the cells, respectively.

5794

Braune, M.O. 1964. Standardization and application of the fluorescent antibody technique in the diagnosis of avian viral respiratory diseases. Diss. Abstr. 24:4914-4915.

FA techniques were developed and standardized for study of the viruses of Newcastle disease, infectious bronchitis, and infectious laryngotracheitis. The conditions for preparation of conjugates and staining are given. Virus was demonstrated in cell cultures. Comparison of the relative diagnostic efficiency of FA and virus isolation indicated that FA was more sensitive in the early disease stages. The methods were equivalent at the peak of the disease, and virus isolation was better near the end. Field diagnoses were successfully attempted by FA.

5795

Braune, M.O.; Gentry, R.F. 1965. Standardization of the fluorescent antibody technique for the detection of avian respiratory viruses. Avian Dis. 9:535-545.

Each phase of the FA technique was investigated. Gamma globulin solutions prepared from specific antisera against Newcastle disease virus, infectious bronchitis virus, and infectious laryngotracheitis virus having neutralization indices of 3.5 or greater were satisfactory for conjugation. The optimum conjugation ratio of FITC to protein was 0.03 mg dye per mg protein at a conjugation time of 12 hours. Reactions of a high fluorescent intensity were observed in infected tracheal smear preparations that were fixed in acetone at -20 C for 10 minutes and reacted for 30 minutes with conjugates adjusted to contain twice the protein concentration of the FA titration endpoint. A washing time of 20 minutes or more in phosphate-buffered saline did not affect the intensity of specific reactions. Infected tracheas could be stored at 4 C under moist conditions for 72 hours and retain their reactivity.

5796

Brown, G.C.; Maassab, H.F.; Veronelli, J.A.; Francis, T.J., Jr. 1964. Rubella antibodies in human serum: Detection by the indirect fluorescent antibody technique. Science 145:943-945.

Antibodies for rubella virus were detected in human serum and titrated by the indirect method of immunofluorescence; a chronically infected, continuous line of monkey kidney cells was used as antigen. Positive reactions were obtained with sera from convalescent patients or persons who had been exposed to the virus. Sera from patients in the acute stage of the disease and those from unexposed individuals were negative.

5797

Brown, G.C.; Maassab, H.F.; Veronelli, J.A.; Francis, T.J., Jr. 1965. Detection of rubella antibodies in human serum by the indirect fluorescent antibody technique. Arch. Ges. Virusforsch. 16:459-463.

Antibodies for rubella virus were detected in human serum and titrated by the indirect method of immunofluorescence using a chronically infected, continuous line of monkey kidney cells as antigen. Positive reactions were obtained with convalescent or postexposure specimens; acute sera and those from unexposed individuals were negative.

5798

Butler, M.; Leach, R.H. 1964. A mycoplasma which induces acidity and cytopathic effect in tissue culture. J. Gen. Microbiol. 34:285-294.

An agent that induced acidity and cytopathic effects in Hep-2 tissue cultures was investigated. The agent grew well in certain other tissue culture systems. Typical mycoplasma colonies were isolated from the contaminated Hep-2 cultures and on reinoculation into Hep-2 cultures produced effects indistinguishable from the original effects. There was no appreciable growth in tissue culture medium alone. The mycoplasma had biological properties similar to those of known mycoplasmas, including Mycoplasma hominis Type 1, but was serologically distinct from these. Fluorescent antibody and Giemsa-staining techniques showed extracellular forms. Other mycoplasmas were shown to grow in tissue culture; M. gallisepticum induced similar effects to the cytopathic agent but was distinct in serological and biological properties. The agent partially inhibited the growth of measles virus.

5799

Chin, T.D.Y.; Welton, E.R.; Liu, C. 1964. Rapid identification of influenza and parainfluenza viruses isolated in tissue cultures by immunofluorescence. Bacteriol. Proc. M221:86.

Direct FA was employed for viruses isolated in primary monkey kidney monolayers. Samples (0.1 ml) of throat washings from patients with respiratory illness were inoculated into each of three cultures. The presence of a hemagglutinating agent was detected by adding 0.2 ml of a 0.4 per cent guinea pig RBC suspension to one of the tubes on the 3rd, 5th, and 10th days. When the cultures showed evidence of hemadsorption, the cells were scraped from the wall of the tube and sedimented by centrifugation. The cells were spread on glass slides and stained with specific FA conjugates of influenza virus Types A-2 and B and parainfluenza virus Types 1, 2, and 3 with appropriate controls.

Of 95 agents specifically identified by FA staining, 32 were Type A-2 influenza virus, 33 Type B influenza virus, 12 Type 1 parainfluenza virus, 4 Type 2 parainfluenza virus, and 14 Type 3 parainfluenza virus. Of 95 viruses identified by FA, 83 were confirmed by HI. The remaining 12 agents could not be tested by HI because of low hemagglutination titers. The nine isolates not specifically stained by the FA conjugates were mumps virus, simian-like agents, or unidentified. There was no false identification by FA; virus could be identified within 2 hours when the specimen showed hemadsorption.

5800

Cole, F.E., Jr.; Hetrick, F.M. 1965. Persistent infection of human conjunctiva cell cultures by Myxovirus parainfluenza 3. *Can. J. Microbiol.* 11:513-521.

A human conjunctiva cell line persistently infected with the SF-4 strain of Myxovirus parainfluenza 3 (PIV-3) was studied. Normal cultures inoculated at a multiplicity of infection of 10 exhibited no cytopathic changes if the cultures were kept in growth medium; the use of maintenance medium did result in culture destruction. The maintenance of the carrier state was dependent, at least in part, on active cell growth and metabolism. During 76 subpassages, FA, hemadsorption, inclusion staining, and cell-cloning procedures indicated infection of 61 to 97 per cent of the cells. The carrier exhibited moderate resistance to superinfection with polio virus and ECHO 6 virus, but showed no resistance to vesicular stomatitis virus, Herpesvirus hominis, or Guaroa virus. Subpassage of a carrier culture for 5 months in antibody-containing growth medium produced a gradual decrease in the proportion of infected cells, although a cure was not effected. This gradual decrease of infected cells suggested an intracellular transfer of the virus during mitosis; this was confirmed by FA and cloning procedures.

5801

Corstvet, R.E.; Sadler, W.W. 1964. The diagnosis of certain avian diseases with the fluorescent antibody technique. *Poultry Sci.* 43:1280-1288.

A modified method of conjugate preparation produced conjugates that were satisfactory for differentiating various avian pathogens in artificial media and tissues. This simplified method saved considerable time over previously published methods by eliminating fractionation of the unconjugated serum, dialysis, and concentration. This was desirable since indirect FA did not function with colony imprints. FA can be very useful in evaluating mycoplasma cultures as to purity and greatly simplifies the identification of Mycoplasma spp. or serological types as well as other avian pathogens. The results also showed that tissue imprints of trachea, air sac, and proventriculus could be used in identifying NDV by FA.

5802

Dinter, Z.; Hermodsson, S.; Hermodsson, L. 1964. Studies on myxovirus Yucaipa: Its classification as a member of the paramyxovirus group. *Virology* 22:297-304.

A newly isolated respiratory virus of chickens, myxovirus Yucaipa (MVY) has been classified as a paramyxovirus. MVY is very similar to Newcastle disease virus but is serologically distinct from it. Indirect FA was used to stain the virus in monkey kidney cells.

5803

Frigan, F.T.; Bird, T.J. 1965. Effect of 5-fluorophenylalanine on Newcastle disease virus and vesicular stomatitis virus in tissue cultures. *Bacteriol. Proc.* V126:118.

The suppression of cytopathic effect, CPE, of Newcastle disease virus, NDV, and vesicular stomatitis virus, VSV, was studied with tolerated doses of a variety of drugs in chicken fibroblast monolayers. Of 30 drugs investigated, only five inhibited the CPE when the monolayers of cells were infected with 1,000 TCID₅₀ of each virus. On the other hand, the only significant inhibition of the synthesis of infectious NDV and VSV was effected by 5-fluorophenylalanine, FPA. NDV-infected cells treated with 30 ug of FPA gave only 7 per cent of control infectivity; the VSV titer obtained under the same circumstances was 15 per cent. In the presence of 30 ug of FPA, the NDV hemagglutinin activity extracted from infected cells was decreased more than fivefold, representing 18 per cent of hemagglutinin obtained from untreated cells. Purine-bound ribose determinations of normal HeLa cells, NDV-infected cells, normal cells treated with FPA, and infected cells treated with FPA showed that FPA inhibits synthesis of RNA in general, and probably, therefore, of protein also; however, the most drastic treatment of the infected cells by FPA, 300 ug per ml of medium, does not completely inhibit the synthesis of viral specific materials within such cells, as shown conclusively by acridine orange and fluorescent antibody microscopy.

5804

Funckes, A.J.; Ly, H.; Gates, E.H.; Imagawa, D.T. 1963. Procedures to decrease nonspecific fluorescent staining of measles-infected tissue. *Federation Proc.* 22:217:197.

Because of problems associated with nonspecific fluorescence in immunofluorescent techniques, a study was made to determine the significance of some physical properties of media. Whole sera were labeled with fluorescein isothiocyanate, which was fractionated by low-temperature

ethanol procedures. Fluorescein-labeled antibody fractionated with glycine-ethanol mixtures contained only traces of free fluorescein. These labeled antibodies are absorbed by normal tissue homogenate to reduce nonspecificity. The effect of pH, ionic strength, and dielectric constant of media during these absorptive procedures was found to be important. During the staining procedure, alteration of pH, ionic strength, and dielectric constant from the procedures used during absorption can significantly affect nonspecific staining. Nonspecific fluorescence was significantly reduced as shown by comparative photomicrography in measles-infected mouse brain smears and in measles-infected HeLa cell cultures. Complete article.

5805

Gamo, I.; Kinoshita, Y.; Kuki, N.; Uehara, Y.; Yoneda, S.; Kurumi, K.; Inada, T.; Toyazawa, S.; Maruyama, G.; Takahashi, M.; Toyoshima, K. 1963. Vaccination with egg passage measles virus by inhalation; especially upon its clinical response and spread to the surroundings. *Acta Paediat. Jap.* 5:10:1-8.

Studies were carried out comparing serological responses of children in measles and in 'vaccination measles' produced by inhalation of an attenuated live virus vaccine. Measles antigen was detected by the direct FA method using FITC in smear preparations taken from the conjunctiva, nose, and Koplik's spots of measles patients, but results were negative in similar preparations of conjunctiva, nose, Koplik's spots and pharynx of 'vaccination measles' patients.

5806

Henle, G.; Henle, W. 1965. Cross-reactions among gamma globulins of various species in indirect immunofluorescence. *J. Immunol.* 95:118-124.

Several preparations of fluorescein isothiocyanate - conjugated rabbit antibodies to guinea pig or human gamma globulin fractions were found to be capable of staining the characteristic mumps virus inclusions in infected HeLa cells previously sensitized with virus-specific antibodies derived from heterologous animal species. Brilliant immunofluorescence was elicited by labeled antibodies to heterologous gamma globulins only when the antiviral sera used for the primary stage of the reaction were used undiluted, or at the most in 10-fold dilution. The rabbit anti-guinea pig gamma globulin sera from which effective fluorescein conjugates were prepared yielded from one to three lines of precipitation in Ouchterlony tests with sera from several heterologous species with the exception of rabbits, whereas those sera from which strictly species-specific conjugates were obtained produced no precipitates with heterologous sera. Cross-reacting antisera to guinea pig gamma globulin also reduced the viral neutralizing capacity of human poliomyelitis convalescent serum. No attempts were made to identify the cross-reacting serum components. Such broadly reactive anti-gamma-

globulins might be useful for the indirect technique of immunofluorescence in that they overcome the usually observed species limitation of this method.

5807

Henle, W. 1963. Interference and interferon in persistent viral infections of cell cultures. *J. Immunol.* 91:145-150.

This paper discusses virus-cell interaction that permits maintenance of chronically virus-infected continuous cell lines. Numbers of infected cells have been assessed by immunofluorescence as well as by other methods. Immunofluorescence is particularly well suited to such an application.

5808

Hermodsson, S. 1963. Inhibition of interferon by an infection with parainfluenza virus Type 3 (PIV-3). *Virology* 20:333-343.

The autointerference of Newcastle disease virus (NDV) in calf kidney cultures is inhibited by a previous infection with PIV-3. PIV-3 also enhances the reproduction of NDV in cultures persistently infected with the latter virus. This effect of PIV-3 appears to be associated with the infectious virus particles and to be due to an inhibition of the production and antiviral action of interferon. Interferon produced by foot-and-mouth disease virus is also inhibited by PIV-3. Indirect FA staining was used to detect viral antigen in calf kidney cells. FA results demonstrated that cells would be doubly infected with these viruses.

5809

Hermodsson, S. 1964. Role of interferon in the autointerference of Newcastle disease virus (NDV). *Acta Pathol. Microbiol. Scand.* 62:133-144.

The autointerference phenomenon that appears in calf kidney cultures infected with NDV has been associated with the presence of interferon in the inoculum. This amount of interferon does not prevent synthesis of viral protein but significantly reduces the virus yields and the cytopathic effect. The inoculation of undiluted NDV suspensions induces a persistent infection that is probably maintained not only by production of interferon but also by a reduced ability of the cells to adsorb virus.

5810

Hers, J.F.Ph. 1963. Fluorescent antibody technique in respiratory viral diseases. Amer. Rev. Resp. Dis. 88:316-338.

Fluorescent antibody diagnosis of respiratory viral disease is rapid and sensitive. It also provides a tool for pathogenesis study. Among the diseases discussed are influenza, Eaton's agent, and psittacosis. The discussion is particularly informative.

5811

Hinuma, Y. 1964. Studies on a carrier state of HA 2 virus in HeLa cell cultures by means of immunofluorescent procedure. Jap. J. Med. Sci. Biol. 17:281.

Mechanism of transfer of viral antigens in a culture of HeLa cells persistently infected with hemadsorption Type 2 virus (HeLa/HA 2) was studied. The HeLa/HA 2 cells formed clones with virtually 100 per cent plating efficiency similar to the uninfected HeLa cells. Cell killing effect of the virus in this experimental condition can completely be denied. All of the cells in all of the clones derived from the HeLa/HA 2 culture apparently bear the specific virus antigen without exception when examined by means of FA. The clones of HeLa/HA 2 cells again can form subclones all possessing the virus antigen. The mixture of HeLa/HA 2 and HeLa cells seeded in a single dish independently formed clones, which were easily differentiated by means of FA. The virus possibly released from HeLa/HA 2 cells does not infect normal HeLa cells. The HeLa/HA 2 clones grown in the medium containing an antiviral serum did not affect the formation of the virus antigen in the cells. Results suggest an intimate interaction of the viral gene with that of the cell. Virus antigen in HeLa/HA 2 cells was transferred to daughter cells through cell division.

5812

Hinuma, Y.; Miyamoto, T.; Ohta, R.; Ishida, N. 1963. Counting of Sendai virus particles by an immunofluorescent technique. Virology 20:405-412.

Sendai virus from L cell harvests was adsorbed to chicken erythrocytes or L cells and fixed smears were stained by the complement immunofluorescent technique. The numbers of the fluorescent particles corresponded linearly to the virus hemagglutinin units adsorbed from suspensions. Excellent correlation was found between the numbers of fluorescent units and the counts of particles adsorbed to chicken red cells as examined by electron microscopy.

5813

Howe, C.; de Vaux St. Cyr, C.; Morgan, C.; Hsu, K.C. 1965. Immunochemical investigation of parainfluenza (Type 2) virus. Federation Proc. 24:1388:379.

The growth cycle of parainfluenza virus (Type 2) was studied in stable amnion cell cultures grown initially in Eagle-basal medium with calf serum and maintained after infection without serum. The appearance of intracellular and extracellular virus was gauged by infectivity and hemagglutinin titrations and by neuraminidase assays. Immuno-electrophoretic analysis of cells at successive stages after infection revealed new precipitating antigens lacking in normal cells. Viral antigen was identified in the cytoplasm and at the cell periphery by immunofluorescence microscopy. Application of ferritin-conjugated antibody to unfixed infected cells permitted electron microscopic identification of viral particles. Complete article.

5814

Ishida, M. 1963. Analysis of the nature of infectious virus disease by the fluorescent antibody method. Nippon Densenbyo Gakkai Zasshi 37:212-218. In Japanese.

The progress of influenza virus PR8 infection in the mouse was followed at the cellular level by the fluorescent antibody method. Virus accumulated in the kidney as well as in the pulmonary epithelium. The diagnosis of fluorescent decidual cells was also applied to Coxsackie B5 and measles infections. Fluorescent antibody is useful in locating the site of contagious virus infections.

5815

Ishida, N.; Homma, M.; Osato, T.; Hinuma, Y.; Miyamoto, T. 1964. Persistent infection in HeLa cells with hemadsorption virus Type 2. Virology 24:670-672.

HeLa-HA2 harbors the viral antigen in each cell and the antigen can be passed to daughter cells through cell division. Since the treatment with antibody failed to cure the carrier state, this state is not dependent on virus in the medium. Indirect and complement FA staining methods were used. Viral inclusion bodies were stained. These corresponded with Giemsa staining bodies.

5816

Jerushalmy, Z.; Kaminski, E.; Kohn, A.; De Vries, A. 1963. Interaction of Newcastle disease virus with megakaryocytes in cell cultures of guinea pig bone marrow. *Proc. Soc. Exp. Biol. Med.* 114:687-690.

Incubation of bone marrow cells with NDV results in morphological changes in megakaryocytes and suppression of thrombopoiesis. Fluorescent antibody staining of infected megakaryocytes in cell culture shows adsorption of NDV, an eclipse period, and formation of new viral antigen starting at 4 hours after infection.

5817

Johnson, C.F.; Scott, A.D. 1964. Cytological studies of Newcastle disease virus (NDV) in Hep-2 cells. *Proc. Soc. Exp. Biol. Med.* 115:281-286.

Hep-2 cover slip cultures were infected with the CG strain of Newcastle disease virus and incubated at 32, 37, and 42 C. Infected cultures contained infected mononucleate and multinucleate cells when stained at frequent intervals with hematoxylin and eosin and with acridine orange stains. It was found that fluorescein-conjugated antibody stain demonstrated the presence of viral antigen in the nucleus and cytoplasm as early as 6 hours after infection in cultures incubated at 37 or 42 C. At 32 C, antigen was demonstrable 12 hours after infection. The nuclear fluorescence was transient, disappearing between 24 and 48 hours after infection. In cover slip cultures stained with hematoxylin and eosin or with acridine orange, infected cells could not be differentiated until 12 hours after infection. These stains revealed the presence of granules, possibly containing RNA, corresponding in position and size to those staining with fluorescent antibody in the perinuclear zone. Syncytia could be classified into two groups. They could differ in origin or be different stages of infection of one type. The cultures incubated at 37 and 42 C could not be differentiated from each other. The appearance of viral antigen as detected with fluorescein-labeled antibody and the cellular alterations seemed to be delayed by approximately 6 hours in infected cultures incubated at 32 C.

5818

Kashiwazaki, H.; Homma, M.; Ishida, N. 1965. Assay of Sendai virus by immunofluorescence and hemadsorbed cell-counting procedures. *Proc. Soc. Exp. Biol. Med.* 120:134-138.

The fluorescent cell-counting technique was successfully extended to the Sendai virus and L cell system. By this technique, the rate of adsorption of Sendai virus onto L cells was determined precisely. The

hemadsorbed cell-counting technique was also devised in this system and compared favorably with the fluorescent cell-counting technique. Because of its simplicity it is considered a useful technique for the determination of infective virus. Evidence was obtained that a single infectious unit of this virus is sufficient to produce an infected cell, determined either by the fluorescent cell- or the hemadsorbed cell-counting technique. The fluorescent cell-counting technique was as sensitive as the ordinary 50 per cent end-point titration method in eggs and its superiority was shown by its reproducibility.

5819

Kashiwazaki, H.; Ishida, N. 1965. Inhibition of Sendai virus by N1-isobutylbiguanide hydrochloride as studied by immunofluorescent staining. *Proc. Soc. Exp. Biol. Med.* 120:139-143.

In the presence of a nontoxic concentration of N1-isobutylbiguanide hydrochloride, HA production in L cells infected with Sendai virus was reduced to one-sixteenth of the control. The effect of the compound was reversible, and the presence of the compound through the entire course of infection was essential for the inhibition. The number of infectious centers was not reduced even when the cell was treated with the compound all through infection, indicating that the compound did not affect the processes of adsorption or penetration of the virus. However, the infected cells remained in the first stage of antigen development in the presence of the compound, i.e., the accumulation of antigens was limited to the juxtanuclear region of cytoplasm and not extended to the entire cytoplasm. It is concluded that the effect of the compound is on the inhibition of the late maturation mechanism of Sendai virus synthesis.

5820

Ketiladze, E.S.; Zhilina, N.N.; Mekler, L.B.; Naumova, V.K.; Lozhkina, A.N.; Orlova, N.N.; Nisevich, L.L. 1964. Use of the fluorescent antibody method for rapid differential diagnosis of influenza, parainfluenza, and adenovirus illnesses. *Vop. Virusol.* 9:348-353. In Russian.

The direct immunofluorescence method with highly purified fluorescent antisera against influenza, parainfluenza, and adenoviruses was employed to detect virus antigen in cells of nasopharyngeal mucosa of patients with acute respiratory illnesses. The preparations were stained in parallel with acridine orange fluorochrome for revealing RNA and DNA inclusions. During an inter-epidemic period the method proved helpful for determining virus etiology of more than 50 per cent of such cases within the first days of illness. A good correlation was found between the results of immunofluorescence and those of serological investigations with paired sera. RNA and DNA inclusions were demon-

strable more often in cases where positive immunofluorescence with a respective serum was seen. During a small influenza B outbreak positive immunofluorescence results with anti-influenza serum were obtained in 75 per cent of patients with influenza and early influenza complications. This is a valuable and highly specific method for early differential diagnosis of acute respiratory virus infections.

5821

Ketiladze, E.S.; Zhilina, N.N.; Mekler, L.B.; Naumova, V.K.; Orlova, N.N.; Lozhkina, A.N.; Nisevich, L.L. 1964. The significance of the fluorescent antibodies method for rapid etiological diagnosis of acute respiratory viral affections in children. Vop. Okhrany Materin. Detstva 9:8:28-33. In Russian.

Results are presented of direct FA method used in examining the nasopharyngeal smears obtained from 120 children with acute respiratory diseases. The use of fluorescent sera containing antibodies against influenza virus, parainfluenza virus, and adenoviruses has made possible identification of 56 per cent of the infections, 14 per cent of which proved to be etiologically connected with influenza, 19 per cent with parainfluenza, and 23 per cent with adenoviral infection. The viral antigen was detectable at any stage of the disease when marked clinical symptoms of the disease were present. Comparison of the immunofluorescence results on the smears and serological and virological investigations demonstrated not only the high specificity of FA, but also ascertained its definite diagnostic advantages. The rapidity and the simplicity of the direct FA method has offered a possibility of obtaining the results of nasopharyngeal smear examinations in 3 hours. This makes this method most promising for clinical practice.

5822

Koffler, D. 1964. Giant cell pneumonia: Fluorescent antibody and histochemical studies on alveolar giant cells. Arch. Pathol. 78:267-273.

Giant cell interstitial pneumonia was diagnosed following an episode of clinical measles. Indirect fluorescent antibody reaction with the patient serum and histochemical stains for RNA were used to localize antigenic material, considered to represent the measles virus, in the nuclear and perinuclear zone within intra-alveolar giant cells.

5823

Lesso, J.; Szanto, J.; Albrecht, P. 1963. Mumps virus infection of HeLa cells studied by the fluorescent antibody method. *Acta Virol.* 7:37-41.

The Enders strain of mumps virus, adapted to the allantois of chick embryo, does not multiply in HeLa cells. Another strain, designated 754, hitherto passaged only in the amniotic cavity of the chick embryo, multiplies and can be serially passaged in HeLa cells. Specific fluorescence could be detected in the cytoplasm early after inoculation of HeLa cells with either strain of mumps virus. Eosinophilic inclusions could be demonstrated in the cytoplasm of HeLa cells inoculated with the 754 strain or with a large dose of the Enders strain. The occurrence of inclusions is not connected with the appearance and amount of antigen in inoculated cells.

5824

Lesso, J.; Voskebova, M. 1965. Diagnosis of mumps and parotitic meningitis by the fluorescent antibody technique. *Acta Virol.* 9:282-283.

Results of attempts at using the fluorescent antibody technique for rapid diagnosis of mumps in human patients are reported.

5825

Liess, B. 1963. Fluorescence serologic studies on cell cultures after infection with rinderpest virus: With a contribution to the problem of a group-specific seroreaction between measles virus and rinderpest antibody. *Zentralbl. Bakteriol.* 190:424-443. In German.

The behavior of two different strains of rinderpest virus in primary calf kidney cells was investigated by the indirect fluorescent antibody technique. Virus antigen production was first detected between the 6th and 12th hours postinoculation in the form of scattered granules that later formed larger aggregates. Marked fluorescence near the cell membrane was found, particularly in spindle-shaped, stellate, or rounded cells. Fluorescent granules within the nucleus were found occasionally in the later stages of infection. In HeLa cells the sites of cytoplasmic fluorescence corresponded with inclusion bodies in preparations that were stained later by the May-Grunwald-Giemsa technique. Measles antigens in HeLa cells showed specific fluorescence after primary treatment with rinderpest-immune serum.

5826

Liess, B.; Plowright, W. 1963. The propagation and growth characteristics of rinderpest virus in HeLa cells. Arch. Ges. Virusforsch. 14:27-38.

After 95 passages in primary calf kidney cultures rinderpest virus proliferated readily in HeLa cells and produced characteristic cytopathic effects. A growth-curve experiment showed that the titer of intracellular virus was consistently 10-fold to 100-fold greater than that of free virus, thus conforming to the pattern usually described for the agents of measles and canine distemper. Whereas HeLa cultures infected with measles virus readily adsorbed erythrocytes of Cercopithecus aethiops (vervet) and Papio sp. (baboon), no hemadsorption was noted in rinderpest-infected cultures. The latter also failed to adsorb erythrocytes of rabbits, guinea pigs, sheep, ox, and dog. FA demonstrated virus in the cells.

5827

Llanes-Rodas, R.; Liu, C. 1965. A study of measles virus infection in tissue culture cells with particular reference to the development of intranuclear inclusion bodies. J. Immunol. 95:840-845.

The sequential morphologic changes of tissue culture cells infected with Edmonston measles were followed by combining infectivity titration, direct immunofluorescent, acridine orange and hematoxylin-eosin staining methods. The initial site of measles virus multiplication appeared to be in the perinuclear region of the cell cytoplasm. As the infection progressed, viral antigen spread into the nucleus, first as small granules, and later coalesced into large masses. During the end stage of infection, the content of measles antigen and nucleic acid decreased markedly, leaving a morphologic residue represented by intranuclear eosinophilic inclusion bodies. In this study the direct immunofluorescent staining method is found to be superior to the indirect technique in demonstrating the antigenic development within the measles-infected cells.

5828

Llanes-Rodas, R.; Liu, C. 1965. Morphological studies by immunofluorescent technic of measles virus infection in tissue culture cells. Federation Proc. 24:2432:571.

Immunofluorescence, acridine orange, and hematoxylin-eosin staining methods were used to study the sequential morphological changes of a continuous line of human amnion (AV3) cells infected with Edmonston strain of measles virus. The initial site of measles virus multiplication appeared to be in the perinuclear region of the cell cytoplasm. During the first

48 hours of infection the spread was probably from cell to cell, as shown by clusters of infected cells scattered in various foci in the cell sheet. During this cell-to-cell spread stage, little virus was liberated into the media as reflected by there being no rise of infectious virus titer. When the infection progressed, a rise in extracellular virus titer, formation of multinucleated giant cells, and increase of viral antigen and nucleic acid were noted. Eventually, the measles antigen spread into the nucleus, first as multiple antigenic dots; later, it coalesced into large antigenic masses. During the end stage of infection, about 12 to 15 days after infection, the content of measles antigen and nucleic acid decreased markedly, leaving a morphological residuum represented by intranuclear and intracytoplasmic eosinophilic inclusion bodies. Complete article.

5829

Maestrone, G. 1963. Demonstration of leptospiral and viral antigens in formalin-fixed tissues. *Nature* 197:409-410.

Modifications of tissue preparation procedures for FA staining are described. These modifications warrant further investigation because of the following practical implications: Elimination of the hazard of handling infectious material; simplification of collection, shipment, and storage of suspected specimens; investigation by FA of specimens submitted for routine histopathological examination; and possibility of a more extensive use of this relatively new tool.

5830

Maestrone, G.; Coffin, D. 1964. Study of Newcastle disease by means of fluorescent antibody technique. *Amer. J. Vet. Res.* 25:217-223.

Detection of Newcastle disease virus (NDV) in sections and smears of experimentally infected embryonating eggs and chicks was achieved by the FA procedure. Viral antigen was detected in the epithelial cells of the larynx and vascular walls of internal organs of chicks 3 hours after experimental intranasal infection, 4 days before the appearance of any visible signs of sickness, and 2 days before demonstration of viremia through egg inoculation. The upper respiratory tract seemed to be the site of election for the early multiplication of the virus. Presence of the virus could be demonstrated in tissue smears and sections kept for 10 days at room temperature.

5831

Matumoto, M.; Saburi, Y.; Aoyama, Y.; Mutai, M. 1964. A neurotropic variant of measles virus in suckling mice. Arch. Ges. Virusforsch. 14:683-696.

Strain Sugiyama, adapted to FL cells after six passages in primary monkey renal cells, acquired the ability to propagate serially in the brain of suckling mice somewhere between the 49th and 76th FL passage. The virus produced a lethal spastic paralysis in about half of mice at the early passages and in almost 100 per cent from the 9th passage on. Extranatural routes of inoculation were ineffective for infection. Histologic changes were found only in the cerebrum, not in the cerebellum or spinal cord, nor in other visceral organs, and consisted of degeneration of nerve cells with or without formation of inclusions characteristic of measles, multinuclear giant cell formation by fusion of nerve cells, and proliferation of glia cells. FA study revealed remarkable specificity of nerve cell involvement. Specific measles antigens were demonstrated predominantly in nerve cells, only rarely in glia cells, but not in other types of cell.

5832

Miyamoto, T.; Hinuma, Y.; Ishida, N. 1965. Intracellular transfer of hemadsorption Type 2 virus antigen during persistent infection of HeLa cell cultures. Virology 27:28-36.

A culture of HeLa cells persistently infected with hemadsorption Type 2 virus (HeLa-HA2) was shown to have a similar plating efficiency to that of uninfected HeLa cells, demonstrating that the infection was not pathologic. Each of the cells from derived clones contained virus antigen when examined by immunofluorescent staining. When mixtures of HeLa-HA2 and normal HeLa cells were seeded in a single dish in the absence of antiviral serum, and allowed to form clones, the immunofluorescent-positive and -negative clones were easily differentiated. The ratio of the antigen-bearing to nonbearing clones was very close to that of the number of HeLa-HA2 and normal HeLa cells in the original mixture, indicating that the virus antigen of HeLa-HA2 cells could not transfer to the normal HeLa cells. When the HeLa-HA2 clones were grown in medium containing specific antiviral serum, the serum did not affect the formation of the virus antigen in the cell. Therefore, the persistent infection was maintained by transfer to daughter cells during cell division.

5833

Nayak, D.P.; Twiehaus, M.J.; Kelley, G.W.; Underdahl, N.R. 1965. Immunocytologic and histopathologic development of experimental swine influenza infection in pigs. *Amer. J. Vet. Res.* 26:1271-1283.

The development of swine influenza (S-15) virus infection in pigs was studied by immunocytologic and histopathologic methods. The infectious process was detected by immunofluorescence 2 to 4 hours after inoculation. Microscopic lesions appeared 6 to 8 hours later. Clinical signs, as well as gross pulmonary consolidation, were apparent only after 24 hours. Evidence indicated that swine influenza is an acute, progressive infection. Small bronchi were infected first. The infection spread to terminal bronchioles, alveoli, large bronchi, and trachea. Polymorphonuclear leukocytes were the predominating cells in bronchial epithelium, bronchial exudate, alveolar septums, and blood vessels in the early stages. They were replaced by mononuclear cells in later phases of the infection. Degeneration and exudative changes of bronchial epithelium occurred in the early phase of infection and were followed by hyperplasia of bronchial epithelium, proliferation of lymph node, and thickening of alveolar septums. Exudative changes were never prominent in alveoli where interstitial pneumonia was the predominant feature.

5834

Nii, S.; Kamahora, J. 1964. Detection of measles viral antigen in the lymph nodes of cynomolgus monkeys infected with measles. *Biken J.* 7:71-73.

Direct FA was used to specifically stain measles virus antigen in frozen sections of lymph nodes of challenged monkeys. Fluorescing cells were seen in marginal sinuses and cortical cords. The virus was also in the germinal centers. Infected cells were sometimes agglutinated in masses. These masses seemed to correspond to measles giant cells. Growth of measles virus undoubtedly occurred in the lymphatic tissues.

5835

Norrby, E.C.J.; Magnusson, P.; Falksveden, L.-G.; Groenberg, M. 1964. Separation of measles virus components by equilibrium centrifugation in CsCl gradients: II. Studies on the large and the small hemagglutinin. *Arch. Ges. Virusforsch.* 14:462-473.

CsCl equilibrium density centrifugation studies have been undertaken with the rapid-sedimenting large hemagglutinin (HA) and slow-sedimenting small HA fractions of measles HA. Nucleic acid determinations by tritium labeling and colorimetric reactions were done. Although noninfectious, HA fractions exerted a rapidly appearing cytopathic effect in tissue cultures. No intracellular viral antigen could be detected by indirect FA in syncytia formed in this way.

5836

Palacios, O. 1965. Cytochemical and fluorescent antibody studies on the growth of measles virus in tissue culture. Arch. Ges. Virusforsch. 16:83-88.

Acridine orange staining, together with specific antibody fluorescence, demonstrated that the measles virus was an RNA virus with early involvement of nucleic acid metabolism in the cytoplasm and the nuclei several hours before the virus-specific antigen could be recognized.

5837

Parfanovich, M.I.; Sokolov, N.N. 1965. Interaction of nucleic acids and antigens in tissue culture cells doubly infected with measles and tick-borne encephalitis viruses, as revealed by fluorescence microscopy. Acta Virol. 9:352-357.

After infecting sheep embryo kidney cell cultures with both tick-borne encephalitis (TBE) and measles viruses, both viruses multiplied, but the levels reached by either virus and the accumulation of their antigens depended on the sequence and intervals at which the two agents were inoculated. In mixed infected cultures, the cytopathic effect (CPE) characteristic of measles virus in the given cells was enhanced; however, there remained areas of cells without a CPE but showing an accumulation of TBE virus antigen and RNA-containing material. Distribution of viral antigens and of nucleic acids differed from that found in cells infected with either virus alone. Measles virus antigen and the substance showing green fluorescence after staining with acridine orange occurred mostly in the cytoplasm and not as agglomerates in the nuclei, as with single measles virus infection. Antigen of TBE virus and the RNA-containing substance occurred not only in the cytoplasm, as with single TBE virus infection, but also as agglomerates in the nuclei.

5838

Paucker, K.; Cantell, K. 1963. Quantitative studies on viral interference in suspended L cells. Virology 21:22-29.

The persistence of interference was studied in exponentially growing cultures of suspended L cells after limited exposure to UV-irradiated Newcastle disease virus (NDVuv) or to interferon. Periodic challenge with vesicular stomatitis virus (VSV) disclosed that the duration of protection was largely independent of the size of the interfering dose, provided that the latter was above a given threshold concentration. Susceptibility of the cultures to viral infection was regained by a stepwise process. Cells noted first had the ability to synthesize viral

protein but no infectious virus; then cells appeared in rapidly increasing numbers and produced infectious virus in reduced quantities. Full infectibility of NDVuv-treated cultures was not recovered until about 10 cell divisions had occurred; the effect of interferon was lost somewhat sooner. Protection against influenza A virus, PR8 strain, and VSV diminished simultaneously. On exposure to optimal concentrations of NDVuv or interferon, total resistance against viral challenge was transmitted to at least 8 to 16 descendant cells in the absence of detectable levels of intra- or extra-cellular interferon. Direct FA was used to determine the percentage of infected cells.

5839

Rapp, F. 1964. Plaque differentiation and replication of virulent and attenuated strains of measles virus. *J. Bacteriol.* 88: 1448-1458.

Plaque formation by strains of measles virus in a stable line of African green monkey kidney cells, BSC-1, is characterized by development of large plaques, larger than 1 mm, within 4 days after inoculation of the cultures with the virulent Edmonston strain. Small plaques, less than 1 mm, develop after inoculation with the attenuated Edmonston strain of virus. Plaque formation by measles virus is not influenced by iododeoxyuridine, cytosine arabinoside, isatinthiosemicarbazone, streptonigrin, actinomycin D, or mitomycin C. The predominant cytopathic effect observed with both strains is the formation of large, multinucleated giant cells. Development of the giant cells is correlated with development of virus antigen and synthesis of infectious virus. Synthesis of virus is similar at 34 and at 37 C. Appearance of intracellular virus precedes release, and is later in the attenuated virus-infected cells than in cells infected with the virulent strain. With the virulent strain, equal concentrations of intra- and extra-cellular virus are found but, with attenuated virus, only a small fraction reaches the extracellular fluids, and more than 95 per cent of the newly synthesized virus remains cell-associated.

5840

Reda, I.M.; Rott, R.; Schafer, W. 1964. Fluorescent antibody studies with NDV-infected cell systems. *Virology* 22:422-425.

Characteristic differences exist between Newcastle disease virus (NDV) and influenza virus in localization of the nucleoprotein within the host cells. Influenza nucleoprotein is found first in the nucleus and later in the cytoplasm; NDV nucleoprotein is exclusively in the cytoplasm. Antisera were prepared against both the inner viral component, nucleoprotein, and the outer spiked surface structure. These specific antisera were conjugated, and direct FA was used to follow development of these antisera in tissue culture cells.

5841

Rodriguez, J.E.; Henle, W. 1964. Studies on persistent infections of tissue cultures: V. The initial stages of infection of L(MCN) cells by Newcastle disease virus. J. Exp. Med. 119:895-922.

The initial stages of infection of L cells with standard Newcastle disease virus (NDV) were analyzed to elucidate steps leading to survival of the cultures and to the indefinite persistence of the infectious process at a low level. Cells were exposed to NDV at varying multiplicities. The monolayer cultures derived from such cells were assayed at intervals for cellular growth rates, percentage of infected cells as determined by immunofluorescence, yields of viral progeny and of interferon, and resistance to superinfection with vesicular stomatitis virus. The percentage of cells calculated to be initially infected on the basis of adsorption data was found to match closely the percentage of immunofluorescent cells resulting from the first infectious cycle. Noninfectious virus particles in the progeny released from NDV-infected cells induce resistance in remaining cells or, if adsorbed simultaneously with infectious virus, abort the intracellular infectious process. In both instances interferon is produced; this may then render additional cells resistant. The noninfectious component is considered an incomplete or defective product of viral replication and not merely thermally inactivated virus. NDV partially or completely inactivated at 37 C induced neither cellular resistance nor synthesis of interferon. The incomplete viral component behaved in all respects like ultraviolet-inactivated NDV except that it was significantly more efficient in inducing interferon synthesis.

5842

Rott, R.; Scholtissek, C. 1963. Investigations about the formation of incomplete forms of fowl plague virus. J. Gen. Microbiol. 33:303-312.

During the formation of incomplete fowl plague virus, normal amounts of viral compounds are synthesized. The S-antigen, however, cannot be demonstrated within the cytoplasm in appreciable amounts by FA. The oligonucleotide pattern and the specific radioactivity of the ribonucleic acid (RNA) of incomplete forms are almost identical with those of standard virus. In contrast to chemically inactivated viruses no indication of multiplicity reactivation was found with incomplete forms. Incomplete forms may be lacking in identical pieces of their genome, but contain that part of their RNA that codes for early protein, S-antigen, and hemagglutinin.

5843

Schafer, W. 1963. Structure of some animal viruses and significance of their components. *Bacteriol. Rev.* 27:1-17.

Three viruses were studied: Maus-Elberfeld (ME), fowl plague (FP), and Newcastle disease virus (NDV). FA was used to follow virus antigen production in several cell lines. Viral antigen was detected as early as 3.5 hours after cell infection. Autoradiographic methods were also used to follow nucleic acid changes. It was confirmed that viral nucleic acid contains all the information that the host cell needs to produce new virus. Certain factors within the host cell aid the virus reproduction process at specific stages. Rapidly synthesized RNA in the cytoplasm simultaneously occupied by viral protein may be involved in producing viral protein.

5844

Schieble, J.H.; Lennette, E.H.; Kase, A. 1965. An immunofluorescent staining method for rapid identification of respiratory syncytial virus. *Proc. Soc. Exp. Biol. Med.* 120:203-208.

A fluorescent antibody method for identification of respiratory syncytial virus is described that yielded results in 24 hours. Ten infectious virus particles per ml of inoculum were detected. Of 75 isolates, 71 were identified correctly as respiratory syncytial virus, the others were other viruses. No strain antigenic differences were detected by FA.

5845

Shedden, W.I.; Emery, J. 1965. Immunofluorescent evidence of respiratory syncytial virus infection in cases of giant-cell bronchiolitis in children. *J. Pathol. Bacteriol.* 89:343-347.

Sections of lung tissue showing giant-cell proliferation in bronchioles were obtained at necropsy from four children who died during an epidemic of respiratory tract infection occurring at different times during a period of 10 years. Immunofluorescence studies showed that these giant cells contain antigen of the respiratory syncytial virus or some closely related antigen. Control lung sections that did not show giant-cell change did not contain this antigen. It is suggested that infection with the respiratory syncytial virus or an antigenically related agent may be a cause of giant-cell bronchiolitis in children.

5846

Sokilov, N.N.; Parfanovich, M.I.; Padeyeva, L.L. 1964. Report I: Study by luminescence microscopy of specific antigen nucleic acid distribution in monkey kidney cells infected with measles virus. Vop. Virusol. 9:96-102. In Russian.

A study of measles virus in monkey kidney tissue culture by fluorescent antibody method and by acridine orange staining has revealed a certain parallelism in the distribution of specific antigen in cells. In the course of experimental measles infection the principal process is observed in nuclei of cells in the form of agglomeration of specifically fluorescent green material. At later stages the specifically fluorescent green material can be seen in the perinuclear area of the cytoplasm. In the course of the infectious process amitotic division is regularly noted, which leads to the formation of syncytial cells.

5847

Szanto, J. 1965. Passaging in chick embryos of Newcastle disease virus from persistently infected HeLa cell cultures. Acta Virol. 9:47-54.

Newcastle disease virus (NDV) passaging in chick embryos is described. The amount of infectious virus in the allantoic fluid, determined by plaque titration in individual egg passages, steadily increased. Hemagglutinating virus levels in the allantoic fluids were zero or low; the same applied to infectious virus levels estimated in suspended chorio-allantoic membrane tissue cultures. Examination of intact cells from persistently infected cultures showed that only 0.5 to 0.8 per cent of the cells of the whole cell population produced infectious NDV. When the cell suspension was diluted in specific hyperimmune serum, plaque formation was inhibited. Certain physical factors such as freezing and thawing, ultraviolet irradiation, and temperature lowered the amount of NDV in persistently infected HeLa cell cultures, in some cases to zero values. In high passage levels of persistently infected cultures, viral antigen was demonstrated by immunofluorescence in only 1 to 5 per cent of the cells, but the cultures remained fully resistant to challenge NDV.

5848

Szanto, J.; Albrecht, P.; Vilcek, J. 1963. Investigations on latent infection in the HeLa cell - Newcastle disease virus system. Acta Virol. 7:297-307.

Four lines of latently infected HeLa cells adapted to human and horse serum with strains Blacksburg and Hertfordshire of Newcastle disease virus (NDV) were obtained. The presence of NDV in cultures of all four

lines was confirmed by specific fluorescent antibodies, by eosinophilic cytoplasmic inclusion bodies, and by positive hemadsorption with guinea pig erythrocytes. Titration methods showed that most of the infectious virus was contained in the cellular fraction of the cultures and that only a small part was present in the culture fluid. No hemagglutinins were detected in latently infected cultures by the hemagglutination test. As demonstrated by fluorescent antibodies, latently infected cultures contained intracellular virus, although the cells were cultivated in the presence of specific antibodies. HeLa cells latently infected with NDV were resistant to the cytopathic effect of NDV and fully susceptible to superinfection with herpes simplex virus. Partial resistance to the cytopathic effect of the Saukett strain of poliovirus was observed, but the cells were susceptible to strains Brunhilda and MEF-1, with which the appearance of the cytopathic effect took longer than in the control cultures only after small inocula.

5849

Wertz, R.K.; Adams, W.R. 1963. Presence of viral antigen in inclusion bodies formed in Ehrlich ascites tumor cells infected with Newcastle disease virus. *Yale J. Biol. Med.* 36:234-240.

The inclusion bodies that form in the Ehrlich ascites tumor cell following infection with Newcastle disease virus were studied by the direct fluorescent antibody technique to determine the presence or absence of viral antigen within. It was demonstrated that they contained material that specifically bound anti-NDV rabbit globulin conjugate. In some cases there appeared to be an increased concentration of antigen at the periphery of the inclusion body. Cytoplasmic staining not associated with identifiable inclusions was also observed, indicating that specific intracytoplasmic viral antigen may not be limited to the inclusions.

5850

Williamson, A.P.; Blattner, R.J. 1965. Immunofluorescence of Newcastle disease virus in paraffin-embedded tissues of early chick embryos. *Proc. Soc. Exp. Biol. Med.* 118:576-580.

Paraffin-embedded tissues have been found to be suitable for immunofluorescent studies of Newcastle disease virus - induced defects in chick embryos inoculated at early stages of incubation. In embryos inoculated at 48 hours of incubation and harvested 16 to 24 hours later, viral antigen was observed in many tissues of the embryo proper and in cells of the extraembryonic membranes. The viral antigen was most concentrated in the organs subject to the virus-induced developmental defects, i.e., in tissues differentiating from the body ectoderm, including lens primordia, auditory vesicles, visceral arch ectoderm,

and olfactory epithelium. It was also found in the visceral arch mesoderm and in the epimyocardium of the heart. However, virus was not restricted to these tissues, but was also observed in some cells of the general body ectoderm and was widely distributed in cells of the chorion and amnion and in the yolk sac endoderm. In late infections the specific viral fluorescence was also seen in mesodermal tissues directly underlying the affected ectoderm. Thus viral antigen was distributed in cells derived from all three of the primary germ layers, ectoderm, endoderm, and mesoderm.

5851

Yershov, F.I.; Yun-De, H.; Gorbunova, A.S. 1963. Investigations into syncytium formation in cultures of stable cell lines infected with parainfluenza 1 virus: IV. Cytochemical studies on the formation of A and B types syncytia. *Acta Virol.* 7:316-321.

The properties of A and B type syncytia formed in Sendai (strain MN) virus-infected angiosarcoma cell cultures were investigated by various histochemical methods. Staining with acridine orange revealed that ribonucleic acids in A type syncytia underwent granulation and gradually disappeared, but in B type syncytia their homogeneous fluorescence was preserved. Staining with phosphine 3R revealed the disappearance of lipids in A type syncytia but persistence of lipid granules in B type syncytia. The fluorescent antibody technique demonstrated differences in the distribution of viral antigen in A and B type syncytia.

5852

Zhdanov, V.M.; Azadova, N.B.; Bukrinskaya, A.G.; Graf, I.A.; Zhantiyeva, Ye.M.; Klimenko, S.M.; Kozlova, I.A.; Konoshch, O.V.; Mekler, L.B.; Mel'nikova, L.A.; Naumova, V.K.; Peterson, O.P.; Sivoshinskiy, D.S.; Stakhanova, V.M.; Stefanov, S.B. 1963. Study of intracellular synthesis of viral components. *Vestn. Akad. Med. Nauk SSSR* 18:5:61-67. In Russian.

The site of synthesis of viral components and its chronological sequence were studied in a number of models. This work yielded a variety of data that apparently reflect a diversity in the structure and chemical composition of the viruses. These studies were carried out on models using myxoviruses and smallpox. Distribution of viral antigens was followed by FA and other methods.

5853

Zhdanov, V.M.; Azadova, N.B.; Uryvayev, L.V. 1965. Topography of synthesis of S and V antigens of Sendai virus. J. Immunol. 94:658-661.

S antigen of Sendai virus is synthesized in the nucleoli of infected cells and is then transported to the cytoplasm. V antigen of Sendai virus is synthesized in the perinuclear zone of the cytoplasm and moves to the periphery of the cell where S and V components are assembled and mature virus particles are formed. Treatment with proflavine does not affect the synthesis of either antigen but prevents the transportation of S antigen from nucleoli to the cytoplasm. Indirect FA was employed in these tests.

IX. REOVIRUS

5854

Dales, S.; Gomatos, P.J.; Hsu, K.C. 1965. The uptake and development of reovirus in Strain L cells followed with labeled viral ribonucleic acid and ferritin-antibody conjugates. *Virology* 25:193-211.

Conjugates were prepared by doubly labeling antibody with both FITC and ferritin, and these were used in the study. Reovirus possesses an outer capsid and a subjacent shell. Cells containing purified virus with labeled RNA were sampled over a 12-hour period. About 14 per cent parental label was transferred to foci of progeny virus production. Virus morphogenesis is associated with mitotic-spindle tubules and a cytoplasmic filamentous component.

5855

Hassan, S.A.; Rabin, E.R.; Melnick, J.L. 1965. Reovirus myocarditis in mice: An electron microscopic, immunofluorescent, and virus assay study. *Exp. Mol. Pathol.* 4:66-80.

Newborn white mice uniformly died with extensive myocarditis 5 to 7 days after intraperitoneal inoculation of reovirus Type 1. Myocardial damage was often so severe that by the 5th postinoculation day it was apparent on gross inspection of the heart. Virus was recovered in relatively high titers from the heart during the period when myocardial injury occurred. Viremia was also present, but titers in the bloodstream remained much below those in the heart. Cytoplasmic inclusion bodies, distinctive of reovirus, were seen on histologic examination of myocardial lesions. Viral antigen, demonstrated by the direct fluorescent antibody method, was observed in and around foci of myocardial necrosis. Cellular necrosis was seen with release of virus into the surrounding tissue. Examples of a less severe cellular injury, focal cytoplasmic degradation, were also encountered.

5856

Hobbs, T.R.; Mascoli, C.C. 1965. Studies of experimental infection of weanling mice with reoviruses. *Proc. Soc. Exp. Biol. Med.* 118:847-853.

A mild to severe respiratory infection was established in mice with reoviruses. In certain animals, the virus was detectable in the respiratory tissues although no evidence of illness was observed. In other animals, respiratory illness was evident early, and became overt terminating with the death of the animals. Fluorescent antibody techniques, both

direct and indirect, correlated with other methods of virus detection. The FA - red cell technique proved to be a rapid, sensitive, and highly specific method of reovirus detection and seems to be applicable for detection of other hemagglutinating viruses.

5857

Joncas, J. 1964. The direct fluorescent antibody technique studied with reovirus Type I. *Rev. Can. Biol.* 23:333-338.

A fluorescent antibody technique is described that consists of a few technical modifications of the standard method. Reovirus Type I was the antigen used. Tissue culture medium devoid of antibiotics and of phenol red has been found to decrease the intensity of the auto-fluorescence of the tissue culture. The length of the staining period has been shortened to 10 minutes at room temperature with improved results. A homemade tray is mentioned that greatly facilitates the handling of the small cover slips, and the Columbia staining dishes have been found most helpful. Finally, photographs were taken without any change in filters, using an Anscochrome 200 daylight color film on a Leitz microscope.

5858

Oie, H.; Soergel, M.; Loh, P.C. 1965. Growth characteristics of reovirus Type 2 in an established human amnion line. *Bacteriol. Proc.* V28:101.

The intracellular development of reovirus Type 2 and its interaction with an established human amnion cell line, RA, have been investigated. Adsorption studies indicate that maximal adsorption of the virus to the RA cell was attained within 1 hour after exposure. Single-cycle growth studies revealed a latent period of 9 hours and maximal yields were reached at 36 hours postinfection. In comparison, the latent period for reovirus Type 3 in the same cell line was 6 hours, with maximal yields attained at approximately 26 hours postinfection. Immunofluorescent and cytochemical examination with acridine orange of virus-induced alterations in the cell during a single growth cycle of reovirus Type 2 revealed the appearance of virus antigen in the perinuclear region as early as 4 hours and a maximal number of cells containing antigen by 9 hours postinfection and the appearance of orthochromatically green-staining perinuclear inclusions at 9 hours postinfection. These inclusions were resistant to digestion by ribonuclease and deoxyribonuclease. Autoradiographic analyses of RNA metabolism in infected cells indicated changes in nuclear RNA activity at about 6 hours postinfection, followed by increases in cytoplasmic RNA activity.

5859

Rabin, E.R.; Hassan, S.A.; Melnick, J.L. 1964. Reovirus myocarditis in newborn mice: An electron microscopic, immunofluorescent, and virus-assay study. *Federation Proc.* 23:180:130.

Newborn Swiss-Webster white mice uniformly died with extensive myocarditis 5 to 7 days after intraperitoneal inoculation of reovirus Type 1. Myocardial damage was often so severe that by the 5th post-inoculation day it was apparent on gross inspection of the heart. Virus was recovered in relatively high titers from the heart, 10 million PFU per gram, during the period when myocardial injury occurred; viremia was also present but titers in the blood stream remained below those in the heart. Viral antigen, demonstrated by the direct fluorescent antibody method, was observed in and around foci of myocardial necrosis. Reovirus particles were numerous and readily visualized by electron microscopy in a distribution similar to that of viral antigen. Both complete and coreless particles were seen measuring 65 to 75 μ in diameter; crystalline arrays were occasionally noted. Virus particles were embedded in granular matrixes located in the cytoplasm of myocytes between myofibrils. Fine strands formed a filamentous network throughout such matrixes. The earliest pathologic changes in myocytes were swelling and vesiculation of mitochondria and dilatation of the sarcoplasmic reticulum. Cellular necrosis was seen with release of virus into the surrounding tissue. Complete article.

5860

Spendlove, R.S.; Lennette, E.H.; Chin, J.N.; Knight, C.O. 1964. Effect of antimetabolic agents on intracellular reovirus antigen. *Cancer Res.* 24:1826-1833.

Intracellular reovirus antigen localizes in the area occupied by the achromatic figure of mitotic cells. In untreated interphase cells, viral antigen forms a filamentous, intracytoplasmic reticulum, but in the presence of certain spindle poisons it consolidates into clumps. The specificity of this latter phenomenon was investigated in this study. Spindle poisons affect intracellular reovirus antigen indirectly by their action on the spindle or on a closely related organelle of similar physicochemical structure. Cells were FA stained for assay of virus infection.

5861

Spendlove, R.S.; Lennette, E.H.; John, A.C. 1963. The role of the mitotic apparatus in the intracellular location of reovirus antigen. *J. Immunol.* 90:554-560.

Type 1 reovirus antigen, demonstrated by fluorescent antibody in dividing human amnion cells (FL), localized in the areas occupied by the spindles and centrioles. The developmental cycle of viral antigen in interphase cells involves the formation of a reticulum in the cytoplasm that shifts into a perinuclear position as the infection progresses. In the presence of mercaptoethanol, colchicine, podophyllin, and vincalcalcin, antimitotic agents known to affect metaphase spindle organization, the network formation is prevented and the antigen is localized as small to large spheres. It is proposed that the spindle material is present throughout the cytoplasm during the interphase of growth as a filamentous reticulum; because of an affinity between spindle components and viral antigen, the antigen is oriented by the spindle into a network-like structure. Studies of this phenomenon have the potential of contributing to a better understanding of many basic problems involved in host-virus interaction and spindle formation.

5862

Spendlove, R.S.; Lennette, E.H.; Knight, C.O.; Chin, J.N. 1963. Development of viral antigen and infectious virus in HeLa cells infected with reovirus. *J. Immunol.* 90:548-553.

Development of viral antigen and infectious virus were followed in HeLa cells infected with Type 1 reovirus. The antigen went through a sequence of four stages determined by shape and intracellular location. Six to 8 hours after infection, antigen appeared in particulate form in the cytoplasm. The particles coalesced to form a reticulum-like structure disseminated throughout the cytoplasm; the reticulum in most cases subsequently became perinuclear. In the final stage, the antigen lost much of its reticular structure and spread confluent throughout the cytoplasm. The cytoplasm began bubbling and cell processes containing viral antigen constricted and pinched off from the cell. Cell-associated virus and viral antigen appeared simultaneously after 6 to 8 hours of infection and increased to a maximal concentration at 24 hours. The rate of virus release was slow and appeared to be related to the bubbling phenomenon. The effect of the location of antigen and its slow release from the cell may be related to the slow spread of virus in plaques.

5863

Spendlove, R.S.; Schaffer, F.L. 1965. Enzymatic enhancement of infectivity of reovirus. J. Bacteriol. 89:597-602.

Enhancement of infectivity by chymotrypsin treatment has been demonstrated with all three types of reovirus, although not in all viral preparations. Enzyme treatment did not produce a simultaneous increase in the hemagglutinating activity of reovirus Type 1. The infectivity of reovirus Type 1, Lang strain, was increased by treatment with chymotrypsin, trypsin, papain, and a filtrate from a culture of a Pseudomonas sp., but not by treatment with pepsin. Sedimentation experiments showed that the potential for enhancement was closely associated with the virus particles themselves. Results of studies involving various sequential treatments with chymotrypsin, and with heat in the presence or absence of 2 M magnesium chloride, were compatible with the interpretation that inhibited virus is resistant to exposure to a temperature of 56 C in the absence of magnesium chloride, whereas activated virus is thermolabile. Activation of reovirus infectivity by heat in the presence of magnesium chloride and by chymotrypsin was not additive. FA demonstrated virus in tissue culture cells.

X. INFLUENZA VIRUS

5864

Albrecht, P.; Blaskovic, D.; Styk, B.; Koller, M. 1963. Course of A2 influenza in intranasally infected mice examined by the fluorescent antibody technique. *Acta Virol.* 7:405-413.

The course of influenza infection in mice with the inhibitor-resistant strain A2-Bratislava-4-57 of influenza virus adapted to the mouse lungs was studied by the fluorescent antibody method. White mice weighing 12 grams were inoculated intranasally with approximately 500,000 mean infective doses of virus in 0.05 ml of undiluted allantoic fluid. Virus multiplication was first detected 6 to 12 hours after inoculation in alveolar cells of the lungs and in terminal bronchioles. At 18 to 24 hours postinfection virus could be demonstrated in large bronchi and the trachea, possibly moving upward by the ciliary beat of the cylindrical epithelium. Starting from the 24th hour after infection scattered epithelial cells became positive in the nasal mucosa of some animals. Alveolar epithelial cells responded to influenza infection in a way different from that of the epithelial lining of the trachea and bronchi. The latter showed rapid necrosis and desquamation and filled the air passages; the alveolar cells, although infected first, showed slowly developing degenerative alterations. No virus multiplication could be demonstrated in proliferating vascular endothelium. Common and distinct features of influenza infections in man and experimental animals are discussed.

5865

Baratawidjaja, R.K.; Hewson, A.; Labzoffsky, N.A. 1963. Application of immunofluorescence in the diagnosis of influenza from throat washings. *Can. J. Microbiol.* 9:563-565.

Indirect FA provides a valuable tool for the rapid diagnosis of influenza from throat washings. In 73 throat washings tested for the presence of Asian influenza virus by egg inoculation and by FA, identical results were obtained by both methods. Although cross-reactions were noted between strains within the type, the strain-specific reaction could be easily distinguished by a greater intensity of the fluorescence. The FA technique has a great advantage over the isolation procedure, since the results of the examination could be reported within 4 to 5 hours after arrival of the specimens in the laboratory. The technique is applicable only when a known type of influenza virus or a new strain antigenically related to the known strains is involved in the outbreak.

5866

Blaskovic, D.; Albrecht, P.; Lackovic, V.; Lesso, J.; Rathova, V.; Styk, B. 1963. Rapid diagnosis of influenza by the fluorescent antibody method. *Acta Virol.* 7:192.

A rapid diagnosis of influenza infection in man was attempted by the fluorescent antibody technique. A2 influenza infection was serologically confirmed in nine of eleven persons. In five of them the cytoplasm of occasional cylindrical epithelial cells showed specific fluorescence with the A1 conjugate and a stronger one with the A2 conjugate. The efficiency of the fluorescent antibody method is lower than that of serological tests but, in limited experiences, seems to equal that of virus isolation experiments.

5867

Blaskovic, D.; Albrecht, P.; Lackovic, V.; Lesso, J.; Rathova, V.; Styk, B. 1963. Use of fluorescent antibody method for the rapid diagnosis of influenza during an epidemic. *Cesk. Epidemiol. Mikrobiol. Immunol.* 12:3:129-139. In Czech.

During the influenza epidemic in Czechoslovakia in 1962 caused by A2 type virus, immunofluorescent diagnosis was carried out. Conjugated globulins against influenza A1, A2, B, B1, and C were mixed and used to stain smears from the nasal mucosa of 11 individuals during the first 3 days of illness. Specific fluorescence was demonstrated in five individuals. Serologic confirmation was obtained in nine of the 11 cases. On the basis of results obtained, the use of this method in rapid diagnosis is recommended.

5868

Chin, T.D.Y.; Welton, E.R.; Liu, C. 1964. Rapid identification of influenza and parainfluenza viruses isolated in tissue cultures by immunofluorescence. *Bacteriol. Proc.* M221:86.

Direct FA was employed for viruses isolated in primary monkey kidney monolayers. Samples (0.1 ml) of throat washings from patients with respiratory illness were inoculated into each of three cultures. The presence of a hemagglutinating agent was detected by adding 0.2 ml of a 0.4 per cent guinea pig RBC suspension to one of the tubes on the 3rd, 5th, and 10th days. When the cultures showed evidence of hemadsorption, the cells were scraped from the wall of the tube and sedimented by centrifugation. The cells were spread on glass slides and stained with specific FA conjugates of influenza virus Types A-2 and B and parainfluenza virus Types 1, 2, and 3 with appropriate controls.

Of 95 agents specifically identified by FA staining, 32 were Type A-2 influenza virus, 33 Type B influenza virus, 12 Type 1 parainfluenza virus, 4 Type 2 parainfluenza virus, and 14 Type 3 parainfluenza virus. Of 95 viruses identified by FA, 83 were confirmed by HI. The remaining 12 agents could not be tested by HI because of low hemagglutination titers. The nine isolates not specifically stained by the FA conjugates were mumps virus, simian-like agents, or unidentified. There was no false identification by FA; virus could be identified within 2 hours when the specimen showed hemadsorption.

5869

Denk, H.; Kovac, W. 1965. Immunofluorescence research on the course of influenza virus multiplication in the mouse lung: I. Research on infection with mouse-adapted virus. Arch. Ges. Virusforsch. 17:641-653. In German.

The experimental influenza pneumonia of the mouse has been investigated with fluorescent antibody against influenza A2 virus. Contrary to the histopathologic findings but in accordance with the situation found in psittacosis, the virus replication starts at the transition from the bronchiole to the alveolar sac. It spreads first to the surrounding alveolar epithelium and considerably later to the bronchi.

5870

Eaton, M.D.; Levinthal, J.D.; Scala, A.R.; Jewell, M.L. 1965. Immunity and antibody formation induced by intraperitoneal or subcutaneous injection of Krebs-2 ascites tumor cells treated with influenza virus. J. Nat. Cancer Inst. 34:661-672.

Incubation of Krebs-2 ascites tumor cells with influenza virus at high multiplicity reduced the tumor-producing capacity of cell suspensions. These virus-treated cells could then be used as an antigen to stimulate resistance to the tumor when given intraperitoneally. An attempt was made to define the optimal conditions of virus treatment for a non-lethal but effective antigen. Since less than ten Krebs-2 cells given intraperitoneally produced lethal ascites tumors in most Swiss mice, immunization with fully viable cells by this route was virtually impossible. Some mice could be given large numbers of cells subcutaneously, with production of immunity to intraperitoneal challenge. Virus-treated cells given subcutaneously failed to immunize. The serum from mice hyperimmunized with Krebs-2 cells gave specific reactions by fluorescein-antibody labeling of surface antigens. With other tumors a reaction was observed with 1 to 5 per cent of cells in a suspension. When complement was present, the labeling of surface antigens of Krebs-2 cells was followed by cytolysis.

5871

Fedova, D.; Zelenkova, L. 1965. The use of the fluorescent antibody method for the rapid identification of the A2 influenza virus: I. The identification of influenza virus in the epithelial cell sediment of allantoic or amniotic fluid of infected chick embryos. J. Hyg. Epidemiol. Microbiol. Immunol. 9:127-134.

The method of identifying the influenza virus by the fluorescent antibody technique in the epithelial cell sediment of allantoic or amniotic fluid is very specific. It permits the identification of the type of influenza virus in the first positive passage on chick embryo when the hemagglutination titer is usually very low. During the A2 type virus influenza epidemic in 1964, 16 strains of influenza virus were identified by this method. All strains were identified in the first embryonal passages. Immune guinea pig sera with a high titer of antibodies against S antigen, and suitably adsorbed, are very sensitive and specific.

5872

Fedova, D.; Zelenkova, L. 1965. The use of the fluorescent antibody method for the rapid identification of the A2 influenza virus: II. The identification of influenza virus in nasal smears by the fluorescent antibody technique. J. Hyg. Epidemiol. Microbiol. Immunol. 9:135-146.

The method of identifying the influenza virus in nasal swabs by FA was successful in 63 per cent. Of 30 patients in whom influenza Type A2 was demonstrated either by isolation of the virus or by a significant increase in antibodies in convalescent sera, specific fluorescence was found in the epithelial cells in 19 cases. The nose swabs of healthy controls, taken outside the epidemic period and of three patients on whom influenza was not demonstrated, were completely negative. Nose swabs were taken on different days from the beginning of the illness from the 1st to the 11th day, two to three swabs from every patient. Specific fluorescence was found not only in the acute stage of the disease but also in swabs taken toward the end of the 1st week. The number of cells with specific positive fluorescence in nasal smears from the acute stage of the illness is relatively greater and identification easier.

5873

Frommhaugen, L.H.; Martins, M.J. 1963. A comparison of fluorescein-labeled gamma globulins purified by Rivanol and DEAE chromatography. *J. Immunol.* 90:116-120.

Preparations of fluorescein-labeled gamma globulins obtained by the Rivanol procedure were found to be comparable to DEAE gamma globulins in recovery of antibody and specificity of fluorescent staining. These preparations were applied to the fluorescent antibody analysis of the V antigens of influenza virus, where it was necessary to use purified, labeled gamma globulins to achieve full specificity. However, in other virus-cell systems immune globulins prepared by ammonium sulfate precipitation, conjugated at low ratios of fluorescein to protein, and passed through a Sephadex column were found to be comparable to the Rivanol and DEAE globulins in specificity and, at times, superior in stainability. In the reverse situation, where essentially no contaminating host materials are present, as with preparations of bacteria, the use of ammonium sulfate globulins conjugated at high F:P ratios seems to afford the greatest intensity of staining.

5874

Hinuma, Y. 1964. Some problems associated with the application of FA technique to the rapid diagnosis of influenza. *Clinic All-Around* (Osaka) 13:1234-1238. In Japanese.

FA technique can be applied successfully to the rapid diagnosis of influenza by staining smears of nasal swab with antibodies conjugated with fluorescein isothiocyanate. Hamster antiserum containing S-antibody produced by intra-nasal infection with either influenza A or B gave satisfactory results.

5875

Huang, J.S.; Bang, F.B. 1964. The susceptibility of chick embryo skin organ cultures to influenza virus following excess vitamin A. *J. Exp. Med.* 120:129-148.

The conversion of chick embryonic epidermis to mucous epithelium by excess vitamin A in organ culture was accompanied by a corresponding change of susceptibility to influenza and vaccinia viruses. Untreated epidermis of 10- to 12-day chick embryos supported the growth of influenza virus in organ cultures and a maximum infectivity titer was reached 2 to 3 days after infection. At the same time, the epidermis showed squamous keratinization, beginning about the 4th day of cultivation. Addition of excess vitamin A to the skin organ culture induced the following changes: mucous metaplasia of the epidermis, increase in the daily

and maximum yield of influenza virus, and decrease in the production of vaccinia virus. The maximum yield of both viruses remained unchanged if vitamin A was introduced at the time of virus inoculation. The yield of influenza virus system was proportionally related to the concentration of vitamin A added 4 or more days before inoculation of virus. Increasing doses of vitamin A had no effect on the short-term growth of influenza virus in tissue cultures of chorioallantoic membrane. The change of virus specificity is not due to excess vitamin A, but appears related to the change of differentiation produced in the organ culture system.

5876

Ishida, N. 1963. Analysis of the nature of infectious virus disease by the fluorescent antibody method. *Nippon Densenbyo Gakkai Zasshi* 37:212-218. In Japanese.

The progress of influenza virus PR8 infection in the mouse was followed at the cellular level by the fluorescent antibody method. Virus accumulated in the kidney as well as in the pulmonary epithelium. The diagnosis of fluorescent decidual cells was also applied to Coxsackie B5 and measles infections. Fluorescent antibody is useful in locating the site of contagious virus infections.

5877

Ishida, N.; Hinuma, Y.; Sekino, K.; Shiratori, T.; Kudo, H. 1963. Experimental human influenza infections studied by means of immunofluorescent staining of nasal smears. *Tohoku J. Exp. Med.* 78:390-397.

Three volunteers were experimentally infected with an amniotic culture fluid of fresh influenza B isolate. Two cases, which previously had low HAI antibody against the used virus, did not develop influenza B antibody, suggesting failure of the virus infection. No specimens examined throughout the course of infection by an immunofluorescence technique showed accumulation of virus antigens. Only one case, which previously had no detectable antibody against influenza B, developed typical signs and symptoms of influenza. At 48 hours after infection, fluorescent cells were detected by the immunofluorescent technique in the preparations from nasal scrubs. Fluorescent cells were found in the nasal scrub specimens up to 120 hours after infection, but none were found in the specimens obtained from nasal washings, throat scrubs, and throat washings throughout the course of infection. First discovery of fluorescent cells was 8 hours before the temperature rise and 48 hours before the first virus isolation.

5878

Ishida, N.; Morizuka, T.; Hinuma, Y. 1964. Virus antigens in kidneys of mice infected with influenza A. *Nature* 201:421-422.

The distribution of influenza A virus after inoculation into mice was studied. FA and infectious virus titers were compared. Following intraperitoneal injection virus may be found in the lung after 6 hours. Later, about 72 hours, virus was found in the kidneys. It was felt that virus in the kidney had its origin in the lung. Kidney infectious virus titer was lower than that in the lung.

5879

Ketiladze, E.S.; Zhilina, N.N.; Mekler, L.B.; Naumova, V.K.; Lozhkina, A.N.; Orlova, N.N.; Nisevich, L.L. 1964. Use of the fluorescent antibody method for rapid differential diagnosis of influenza, parainfluenza, and adenovirus illnesses. *Vop. Virusol.* 9:348-353. In Russian.

The direct immunofluorescence method with highly purified fluorescent antisera against influenza, parainfluenza, and adenoviruses was employed to detect virus antigen in cells of nasopharyngeal mucosa of patients with acute respiratory illnesses. The preparations were stained in parallel with acridine orange fluorochrome for revealing RNA and DNA inclusions. During an inter-epidemic period the method proved helpful for determining virus etiology of more than 50 per cent of such cases within the first days of illness. A good correlation was found between the results of immunofluorescence and those of serological investigations with paired sera. RNA and DNA inclusions were demonstrable more often in cases where positive immunofluorescence with a respective serum was seen. During a small influenza B outbreak positive immunofluorescence results with anti-influenza serum were obtained in 75 per cent of patients with influenza and early influenza complications. This is a valuable and highly specific method for early differential diagnosis of acute respiratory virus infections.

5880

Ketiladze, E.S.; Zhilina, N.N.; Mekler, L.B.; Naumova, V.K.; Orlova, N.N.; Lozhkina, A.N.; Nisevich, L.L. 1964. The significance of the fluorescent antibodies method for rapid etiological diagnosis of acute respiratory viral affections in children. *Vop. Okhrany Materin. Detstva* 9:8:28-33. In Russian.

Results are presented of direct FA method used in examining the nasopharyngeal smears obtained from 120 children with acute respiratory diseases. The use of fluorescent sera containing antibodies against influenza virus, parainfluenza virus, and adenoviruses has made possible

identification of 56 per cent of the infections, 14 per cent of which proved to be etiologically connected with influenza, 19 per cent with parainfluenza, and 23 per cent with adenoviral infection. The viral antigen was detectable at any stage of the disease when marked clinical symptoms of the disease were present. Comparison of the immunofluorescence results on the smears and serological and virological investigations demonstrated not only the high specificity of FA, but also ascertained its definite diagnostic advantages. The rapidity and the simplicity of the direct FA method has offered a possibility of obtaining the results of nasopharyngeal smear examinations in 3 hours. This makes this method most promising for clinical practice.

5881

Nayak, D.P. 1965. Epizootiology of swine influenza. Diss. Abstr. 26:1292.

The complex etiology of swine influenza involves a pneumotropic virus and Haemophilus influenzae suis. The object of this investigation was (1) to determine the progressive pathogenesis of swine influenza virus (S-15) in its experimental and natural host by FA and histopathological technique; (2) to evaluate the possible synergistic relationship between S-15 virus and Ascaris suum; (3) to detect the virus in swine lungworms. Progressive pathogenesis of S-15 virus in mice intranasally inoculated was followed by direct FA. The conjugate absorbed with swine liver powder and refractionated from DEAE cellulose column, was highly specific and sensitive for S-15 virus. In mice the infected cells were first detected 8 hours postinoculation, cytoplasmic and occasionally nuclear. FA reached a peak on the 2nd and 3rd day, spread to alveoli on the 2nd day, diminished after the 4th day, and disappeared by the 7th. Swine influenza virus progressively infects bronchi, bronchioles, alveolar ducts, and alveoli. Concentration of virus in the inoculum affected the distribution, progression, and development of S-15 virus in mice inoculated intranasally. The time for appearance in mouse lungs decreased with increased concentration of virus. Egg infectivity titer and FA of virus in lungs correlated well. In specific pathogen-free pigs inoculated intranasally with S-15 virus, virus was detectable 2 to 4 hours postinoculation by FA. Clinical symptoms and pathology are described. Coinfection with migrating Ascaris larvae and S-15 virus had a synergistic effect in inducing enhanced mortality in mice, optimally when given 8 days following Ascaris inoculation. Ascaris migration appeared to break the blood-lung barrier for influenza virus. Infectious virus could not be detected in the lungworm extract in pigs or mice stressed with A. suum larvae.

5882

Nayak, D.P.; Kelley, G.W.; Underdahl, N.R. 1965. The effect of varied inoculums on the distribution and progression of influenza virus (S-15) in lungs of mice. Amer. J. Vet. Res. 26:984-990.

Various concentrations of virus in inocula given intranasally to mice affected the distribution and rate of development of the influenza virus (S-15) in the lungs. Undiluted and four tenfold dilutions of infective allantoic fluid were used in these experiments. With undiluted virus the immunofluorescence appeared concurrently in the alveolar and bronchial epithelia, whereas, with the inoculation of diluted preparations, the bronchial epithelium was the initial site for appearance of immunofluorescence that spread progressively and eventually infected the alveoli. The time required for the initial appearance of immunofluorescence decreased progressively with the increased concentrations of virus in the inocula. Titration of virus in the lungs of mice corresponded with immunofluorescence observation. No significant change in the sequence of appearances of immunofluorescence in the cytoplasm or in the nucleus of cells was caused by varying the virus concentrations in the inocula.

5883

Nayak, D.P.; Kelley, G.W.; Young, G.A.; Underdahl, N.R. 1964. Progressive descending influenza infection in mice determined by immunofluorescence. *Proc. Soc. Exp. Biol. Med.* 116:200-206.

A specific immunofluorescent technique was developed for swine influenza virus, using fluorescein-conjugated immune globulins. In studies of the progressive pathogenesis of virus in mice, immunofluorescence was detected in bronchial epithelium 8 hours after inoculation. Fluorescence, at this stage, was mostly cytoplasmic; however, both cytoplasmic and nuclear fluorescence occurred in a few cells. It reached a peak on the 2nd and 3rd day in the bronchi and usually spread to alveoli by the 2nd day postinoculation. After the 4th day postinoculation, it diminished in the alveoli and bronchi and disappeared after the 7th day. It indicated that both cytoplasm and nuclei of infected cells are involved in the synthesis of viral antigens. It was limited to macrophages and epithelial cells of bronchi, alveoli and turbinates. Influenza virus progressively infected bronchi, bronchioles, alveolar ducts, and alveoli during the course of infection.

5884

Onodera, T.; Hinuma, Y.; Ishida, N. 1964. Ineffectiveness of antibody on the intracellular development of influenza virus WS strain in Ehrlich ascites cells in mice. *J. Immunol.* 92:648-656.

Multiplication of this virus in Ehrlich ascites tumor cells was examined in detail by fluorescent antibody staining of the virus antigens, titration of egg infectivity of the cell-associated virus, and counting of cells to measure the oncolytic effect of the virus. By immunofluorescence, intracellular development of the virus was demonstrated both in nucleus and cytoplasm and was found to proceed in a uniform manner

in all cells of a suspension. When virus infection was established in vitro, the oncolytic effect of the virus was not prevented when the cells were implanted in immune mice. Immunofluorescent staining revealed that the development of virus antigens in cells inoculated into immune mice proceeded as in nonimmune mice. Growth curves of the cell-associated virus were identical in immune mice and nonimmune mice. When suspensions of cells grown in immune mice were washed free of antibody, virus multiplication was equal to that of cells from nonimmune mice. The results obtained indicate that there is no inhibitory effect of antiviral antibody on the intracellular processes producing multiplication of WS virus.

5885

Paucker, K.; Cantell, K. 1963. Quantitative studies on viral interference in suspended L cells. *Virology* 21:22-29.

The persistence of interference was studied in exponentially growing cultures of suspended L cells after limited exposure to UV-irradiated Newcastle disease virus (NDVuv) or to interferon. Periodic challenge with vesicular stomatitis virus (VSV) disclosed that the duration of protection was largely independent of the size of the interfering dose, provided that the latter was above a given threshold concentration. Susceptibility of the cultures to viral infection was regained by a stepwise process. Cells noted first had the ability to synthesize viral protein but no infectious virus; then cells appeared in rapidly increasing numbers and produced infectious virus in reduced quantities. Full infectibility of NDVuv-treated cultures was not recovered until about 10 cell divisions had occurred; the effect of interferon was lost somewhat sooner. Protection against influenza A virus, PR8 strain, and VSV diminished simultaneously. On exposure to optimal concentrations of NDVuv or interferon, total resistance against viral challenge was transmitted to at least 8 to 16 descendant cells in the absence of detectable levels of intra- or extra-cellular interferon. Direct FA was used to determine the percentage of infected cells.

5886

Szczygielska, J. 1964. The course of influenza virus infection in mice: II. Lung and liver tissue tested by fluorescent antibody techniques. *Med. Dosw. Microbiol.* 16:35-39. In Polish.

A virus accumulation was found in liver tissue by indirect immunofluorescence. A particularly high affinity of the virus to the vascular endothelium demonstrated itself by the strongest fluorescence observed. The virus may penetrate the walls of blood vessels and pass into the liver tissue. This was evidenced by disperse fluorescence in perivascular areas.

5887

Tateno, I.; Kitamoto, O. 1965. Rapid diagnosis of influenza by means of fluorescent antibody technic: III. Some new aspects of influenza virus infection revealed by fluorescent antibody technic. Jap. J. Exp. Med. 35:411-417.

The influenza virus antigen was detected in the nasal epithelial cells of seven cases obtained as long as 8 to 23 days after the onset of acute influenzal attack. All these cases except one were febrile. The influenza virus antigen was also detected in the buffy coat of heparinized blood of a patient in the acute stage with high fever, in the eye secretion of two cases, and in the conjunctival cells of a case. The conjunctivae were injected in all these latter three cases. The buffy coat of heparinized blood and eye secretions may serve as a useful material of influenza virus containing cells in the immunocytological diagnosis of influenzal infection.

5888

Tateno, I.; Kitamoto, O.; Makino, M.; Takeuchi, Y.; Sonoguchi, T. 1965. Rapid diagnosis of influenza by means of fluorescent antibody technic: II. Relation between immunocytologic, serological, and clinical findings. Jap. J. Exp. Med. 35:401-410.

The definite diagnosis of influenza virus infection was made in 89 per cent of serologically positive and in 68 per cent of serologically negative patients of acute respiratory disease during the epidemic of influenza A2 by means of direct fluorescent antibody staining of the nasal smears. Less intense infection of the nasal epithelium was observed in the serologically negative than in positive patients. All the patients with temperatures of over 39 C and about 80 per cent of those under 38.9 C yielded positive smears. Two-thirds of the patients positively diagnosed by FA were diagnosed as influenza. The remaining patients were diagnosed as influenzal pneumonia, febrile and afebrile respiratory illness, and common cold. Positive smears were usually obtained within 3 days of the onset of illness but some cases yielded positive smears in their 2nd or 3rd week of the illness.

5889

Tateno, I.; Suzuki, S.; Nakamura, S.; Kawamura, A., Jr. 1965. Rapid diagnosis of influenza by means of fluorescent antibody technic: I. Some basic information. Jap. J. Exp. Med. 35:383-400.

Type-specific immune sera against influenza A2 and B viruses prepared in the guinea pigs were conjugated with FITC. The chiefly fluorescent cells from nasal smears were ciliated and unciliated (polygonal and round in shape) epithelial cells, denuded nuclei and deformed, shrunk, or condensed untypable cells. The fluorescence was located in the nuclei including the denuded nuclei, in both cytoplasm and nucleus, and in the cytoplasm alone. Of the definitely positive specimens, one-half contained many, one-third contained several, and the remainder contained a few positively stained cells. The unstained and unfixed nasal smears can be stored for at least a month at -20 C without significantly deteriorating the stainability of the infected cells.

5890

Zhilina, N.N.; Ketiladze, E.S.; Mekler, L.B.; Orlova, N.N.; Lozhkina, A.N. 1964. Early diagnosis of influenza by the fluorescent antibody method. Sovet. Med. 28:6:85-90. In Russian.

The direct method of immunofluorescence using purified influenza fluorescent serum is highly specific. It makes possible the detection of antibodies in the nuclei and cytoplasm of the columnar epithelium of the mucous membrane in the nose of influenza patients from the first days of illness. During an epidemic rise in morbidity and during the inter-epidemic period, direct FA makes it possible to confirm a diagnosis of influenza in the same percentage of cases as do serological methods. The rapidity of results with the direct method makes it advantageous to recommend this method for clinical practice.

XI. OTHER VIRUSES

A. OTHER ANIMAL VIRUSES AND GENERAL STUDIES

5891

Aiken, J.M.; Hoopes, K.H.; Stair, E.L.; Rhodes, M.B. 1964. Rapid diagnosis of hog cholera: A tissue-impression fluorescent antibody technique. J. Amer. Vet. Med. Ass. 144:1395-1397.

A rapid, simple fluorescent antibody technique performed with tissue impressions was found useful for diagnosing hog cholera. The technique employs a minimum of equipment and specialized procedures and can be conducted in less than an hour. Specificity and accuracy of results obtained with this technique compared favorably with specificity and accuracy obtained with fluorescent antibody techniques performed with frozen tissue sections. Positive test results were obtained with tissues from 22 pigs experimentally inoculated 5 to 16 days previously and with tissues from 10 pigs with naturally occurring hog cholera.

5892

Baratawidjaja, R.K.; Morrissey, L.P.; Labzoffsky, N.A. 1965. Demonstration of vaccinia, lymphocytic choriomeningitis and rabies viruses in the leucocytes of experimentally infected animals. Arch. Ges. Virusforsch. 17:271-279.

During the stage of viremia, viral antigen was demonstrable by means of immunofluorescence in the leukocytes of peripheral blood of experimental animals infected with vaccinia, lymphocytic choriomeningitis, and rabies viruses. Specificity of the fluorescence observed was confirmed in each case by re-isolation of the virus from washed leukocytes by animal or tissue culture inoculation and the identity of re-isolated virus was established by neutralization or immunofluorescence techniques. Possibility of employing FA for early diagnosis of viral infections is discussed.

5893

Barinsky, I.F. 1964. Agglutination reaction mechanism of chicken erythrocytes with the sera of patients with epidemic hepatitis. Zh. Mikrobiol. Epidemiol. i Immunobiol. 41:3:35-39. In Russian.

An anaphylactic reaction was used to ascertain the origin of an agglutinin on chicken red blood cells in infectious hepatitis. There was no agglutinin found in sera from patients with Botkins disease. The agglutination was shown to be nonspecific by various methods including FA. An anaphylactic reaction demonstrated a sensitizing agent in patient bile and serum.

5894

Benda, R.; Hronovsky, V.; Cerva, L.; Cinatl, J. 1965. Demonstration of lymphocytic choriomeningitis virus in cell cultures and mouse brain by the fluorescent antibody technique. *Acta Virol.* 9:347-351.

The indirect fluorescent antibody technique was used to demonstrate lymphocytic choriomeningitis (LCM) virus in cultures of L cells and in mouse brain impression smears. Fluorescent areas specific for the infection were found in impression smears from the brains. Fluorescent antibody technique was found little sensitive and unsuitable for detecting anti-LCM antibody. By immunofluorescence techniques it was possible to follow the production of LCM virus antigen in cells in vitro, to identify cells susceptible to virus infection in the mouse brain, and to demonstrate the virus in macrophages. The results obtained form a basis for the elaboration of a direct one-step technique of immunofluorescence in LCM infection.

5895

Boss, J.H.; Jones, W.A. 1963. Hepatic localization of infectious agent in murine viral hepatitis. *Arch. Pathol.* 76:4-8.

The localization of the infectious agent of murine viral hepatitis was studied with the use of FA. Specific fluorescence in the viral-infected livers was found lining sinusoids, in necrotic foci, and less commonly in hyaline masses occluding sinusoids. This fluorescence is believed to be indicative of foreign antigen, consisting of virus alone or of virus and viral-altered hepatic tissue.

5896

Braune, M.O. 1964. Standardization and application of the fluorescent antibody technique in the diagnosis of avian viral respiratory diseases. *Diss. Abstr.* 24:4914-4915.

FA techniques were developed and standardized for study of the viruses of Newcastle disease, infectious bronchitis, and infectious laryngotracheitis. The conditions for preparation of conjugates and staining are given. Virus was demonstrated in cell cultures. Comparison of the relative diagnostic efficiency of FA and virus isolation indicated that FA was more sensitive in the early disease stages. The methods were equivalent at the peak of the disease, and virus isolation was better near the end. Field diagnoses were successfully attempted by FA.

5897

Hampton, E.G. 1963. Localization of viral antigen in rat embryo cells in tissue culture infected with H-1 virus. Federation Proc. 22:2372:557.

In rat embryo cells infected with H-1 virus, viral antigen developed first in the host cell nuclei and later in the cytoplasm. The direct method of Coons for staining with fluorescent antibodies was used. The FA was obtained from the globulin fraction of hamster serum. After an eclipse period of less than 24 hours, the stain revealed a diffuse fluorescence that occupied the whole nucleus except for areas corresponding in number and size to the nucleoli. On the second day postinoculation the whole nucleus showed fluorescence. At this stage acridine stain revealed nuclear inclusions of DNA-like material. Treatment with nucleases pointed to a protein-bound DNA as a major constituent of the inclusion bodies. Phase microscopy also showed nuclear alterations. The production of inclusion bodies was immediately followed by the release of infective hemagglutinating virus. By the 3rd day, when the viral antigen was present in most of the cells, the inclusion bodies increased in number and the cytopathic effect was appreciable. Complete article.

5898

Hanson, L.A.; Tan, E.M. 1965. Characterization of antibodies in human urine. J. Clin. Invest. 44:703-715.

A number of antibodies in urine were studied, including antibodies against T2 phages, blood group B substance, and antinuclear antibodies in patients with connective tissue disease. The main portion of the antibody activity resided in the 7S gamma globulin; weak activity was noted in the low-molecular-weight fraction related to gamma globulin (gamma-L globulin) in spite of evidence that the 7S gamma globulin was present in smaller amounts than the gamma-L globulin. The low-molecular-weight gamma globulin or urine consisted largely of material identical or similar to L chains, but some material partially identical to H chains of 7S gamma globulin was consistently found. Evidence indicated that the latter finding was not due to contamination with whole gamma globulin. Absorption experiments with antisera showed that this portion of the H chain was essential for antibody activity of the gamma-L globulin fraction, since no activity was present when only the L chains were present.

5899

Ishizaki, R.; Shimizu, T. 1964. Sequential development of antigens of equine rhinopneumonitis virus - infected hamster liver cells as studied with fluorescent antibodies. *Nat. Inst. Anim. Health Quart. Tokyo* 4:194-204.

The development of V and S antigens in hepatic cells of hamsters infected with rhinopneumonitis virus was studied by fluorescent antibody. During the latent period, S antigen was first detected and developed exclusively in the nucleus, whereas V antigen appeared in the cytoplasm with the increase of infectivity and complement-fixing antigens in the infected liver. As the virus multiplication proceeded, the whole cell was filled with both antigens, followed by the disappearance of antigenic materials from the cells, except those in the peripheral area in some hepatic cells, at the terminal stage of infection. The development of inclusion materials as revealed by hematoxylin and eosin staining was closely related to the increase of virus titer as well as the development of viral antigen. In infected hepatic cells, S antigen may be first synthesized in the nucleus, V antigen then synthesized in the cytoplasm, and a mature virus particle may be finally completed in the cytoplasm by assembly of both antigenic materials. S antigen may represent DNA protein and V antigen may constitute the virus particle surface of protein nature.

5900

Ishizaki, R.; Shimizu, T.; Matumoto, M. 1963. Sequential development of antigens of equine rhinopneumonitis virus in cultured horse kidney cells as studied with fluorescent antibodies. *Arch. Ges. Virusforsch.* 12:346-362.

The development of V and S antigens in horse kidney cells infected with equine rhinopneumonitis virus was studied. During the latent period, S antigen was first detected and developed exclusively in the nucleus, whereas V antigen appeared in the cytoplasm with increase in intracellular infectivity. As the virus multiplication proceeded, the whole cell was filled with both antigens, followed by the disappearance of antigenic materials from the cells except the peripheral area at the terminal stage of infection. The development of inclusion materials was closely related to the increase of virus titer as well as the development of the viral antigens. In the infected cells S antigen is synthesized in the nucleus, followed by the synthesis of V antigen in the cytoplasm, and finally the mature virus particle is completed in the cytoplasm by assembly of both antigenic materials, and that S antigen represents DNA-protein constituting the viral core and V antigen constitutes the virus particle surface of protein nature.

5901

Johnson, G.D.; Holborow, E.J. 1963. Immunofluorescent test for infectious mononucleosis. *Nature* 198:1316-1317.

The demonstration of glandular fever antibodies by immunofluorescence affords a convenient method of examining large numbers of sera, and will therefore be of value in epidemiological studies.

5902

Karasszon, D.; Bodon, L. 1963. Demonstration of the swine-fever virus in tissue culture by immunofluorescence. *Acta Microbiol. Acad. Sci. Hung.* 10:287-291.

Swine fever virus was propagated in embryonic pig kidney cell cultures and the virus antigen was demonstrated by indirect FA. Trypsinized suspension of renal epithelial cells was distributed in Legroux flasks, on the bottom of which small slides had been placed. The cultures were infected with the wild virus of swine fever and, after variable periods of incubation, treated with rabbit immune serum prepared against the lapinized strain of swine fever virus. Subsequently, the preparations were treated with anti-rabbit gamma globulin serum produced in goat and labeled with FITC, and examined. An intensive cytoplasmic fluorescence was observed in contrast with the control preparations showing no characteristic fluorescence. The cytoplasmic fluorescence was associated with the antigen of the swine fever virus, which was thus visualized by indirect FA.

5903

King, D.A.; Croghan, D.L. 1965. Immunofluorescence of feline panleucopenia virus in cell culture: Determination of immunological status of felines by serum neutralization. *Can. J. Comp. Med. Vet. Sci.* 29:85-89.

Utilizing FA as a viral indicator for a cell culture serum-neutralization system, a relationship was demonstrated between specific antibody titer and susceptibility of felines to feline panleucopenia virus. This correlation was established by comparing prechallenge serum-neutralization titers to the clinical response of felines challenged with feline panleucopenia virus. The serological and clinical response of felines inoculated with commercial vaccines or related viruses was also studied.

5904

Mallucci, L. 1965. Observations on the growth of mouse hepatitis virus (MHV-3) in mouse macrophages. *Virology* 25:30-37.

Information about some aspects of the biology and nature of mouse hepatitis virus (MHV-3) was provided by using mouse macrophages as an in vitro system. The infection develops fully in 2 or 3 days. The cytopathic effect is accompanied by formation of giant cells that fuse to form a syncytium. Sensitive and rapid methods for titrating the virus were developed, using either direct microscopical examination of unstained cultures or a microfocus assay based on neutral red uptake and concentration in giant cells. Using the indirect method of fluorescent antibody staining, viral antigen was found in the cytoplasm of infected macrophages. The results of growth curves in the presence of metabolic inhibitors of DNA synthesis, FUDR, and actinomycin D suggest that MHV-3 is an RNA virus with no DNA-dependent replicative phase. Although the over-all picture of murine hepatitis viruses is incomplete, the findings suggest that they might belong to the myxovirus group.

5905

Malmquist, W.A.; Fernelius, A.L.; Gormanst, D.E. 1965. Interference of bovine viral diarrhea virus by hog cholera virus in swine kidney cell cultures. *Amer. J. Vet. Res.* 26:1316-1327.

A strain of bovine diarrhea virus, NADL-MD, was adapted to primary and a subculturable cell line of swine kidney cell cultures. The adaptation was followed by comparative titrations in embryonic bovine kidney cell cultures and swine kidney cell cultures and by immunofluorescence. A modified strain of hog cholera virus used to infect swine kidney cell cultures interfered with the cytopathic effect and virus yield of cell-adapted NADL-MD virus. Interference by modified hog cholera virus was dosage dependent and required infection of cells before interference was expressed. Interferon prepared by a conventional method played no role in interference. A swine-passaged strain of hog cholera virus did not produce interference as completely as the strain passaged in swine kidney cell cultures. Early infection of the swine kidney cell line with modified hog cholera virus abolished the cytopathic effect and suppressed the yield of adapted NADL-MD virus.

5906

Mengeling, W.L.; Gutekunst, D.E.; Fernelius, A.L.; Pirtle, E.C. 1963. Demonstration of an antigenic relationship between hog cholera and bovine viral diarrhea viruses by immunofluorescence. Can. J. Comp. Med. Vet. Sci. 27:162-164.

Specific staining of antigen within bovine embryo kidney tissue culture cells, infected with either Oregon C24V or NADL-MD bovine viral diarrhea virus, was accomplished using fluorescein-conjugated swine anti-hog cholera or bovine antiviral diarrhea globulin. Specific staining of antigen within pig kidney tissue culture cells, infected with hog cholera virus, was accomplished using the same two types of conjugates. Specificity was confirmed by appropriate controls. FA was a convenient and sensitive method for determining an antigenic relationship between hog cholera and bovine viral diarrhea viruses.

5907

Mengeling, W.L.; Pirtle, E.C.; Torrey, J.P. 1963. Identification of hog cholera viral antigen by immunofluorescence: Application as a diagnostic and assay method. Can. J. Comp. Med. Vet. Sci. 27:249-252.

In the absence of detectable cytologic changes in hog cholera virus - infected tissue culture cells, hog cholera viral antigen was readily detected by immunofluorescence. The ability to detect hog cholera viral antigen by this method allowed for determination of infectivity titers and also for titration of homologous antibody. FA made possible the identification, in tissue culture, of hog cholera virus from blood, serum, and spleen extracts of experimentally infected swine.

5908

Mirchamsy, H.; Taslimi, H. 1964. Visualization of horse sickness virus by the fluorescent antibody technique. Immunology 7:213-216.

Horse sickness virus was grown in tissue cultures of monkey kidney cells and was detected by a direct fluorescent antibody technique. Virus was detected at 8 hours in or around the nucleus of cells, and at 24 and 48 hours after infection it was also seen in the cytoplasm.

5909

Prince, A.M.; Fuji, H.; Gershon, R. 1964. Immunohistochemical studies on the etiology of anicteric hepatitis in Korea. Amer. J. Hyg. 79:365-381.

FA studies employing the complement staining technique have been carried out with biopsies from cases of anicteric hepatitis detected by mass serum enzyme survey. An antigen was detected in foci of hepatic parenchymal cells in 9 of 32 cases examined, located either in the nuclei, or the cytoplasm, or both. Demonstration of this antigen by the complement staining technique required heat-labile factors in serum. Human serum heat-labile components were preferable to guinea pig serum heat-labile components in this regard. Antibody to the demonstrated antigens was present in a pool of serum from icteric cases of acute viral hepatitis in Korean Army troops, in sera of all cases showing the antigen, and in the serum of one case of probable serum hepatitis. Antibody was not detectable in a variety of control sera. Antibody did not stain control human liver tissues. Cases in which antigen was demonstrated were uniformly of a chronic active type. Antigens observed possibly represented serum hepatitis virus.

5910

Robertson, A.; Bannister, G.L.; Boulanger, P.; Appel, M.; Gray, D.P. 1965. Hog cholera: V. Demonstration of the antigen in swine tissues by the fluorescent antibody technique. Can. J. Comp. Med. Vet. Sci. 29:299-305.

FA was employed to detect hog cholera virus in tissue sections of various organs from experimentally infected swine. The method proved to be highly sensitive and infection could be detected in these animals as early as 3 days after inoculation with the virus. Best results were obtained when tissues were collected from young animals in advanced disease rather than from sows or from pigs in the early febrile phase. Tonsil, spleen, and lymph node were the tissues of choice and were most satisfactory when removed from freshly killed animals rather than from those that had died.

5911

Robertson, A.; Greig, A.S.; Appel, M.; Girard, A.; Bannister, G.L.; Boulanger, P. 1965. Hog cholera: IV. Detection of the virus in tissue culture preparations by the fluorescent antibody technique. Can. J. Comp. Med. Vet. Sci. 29:234-241.

FA was employed for detection of hog cholera virus in tissue cultures inoculated with spleens of infected animals. As controls, cultures were also inoculated with material from normal swine and from those

infected with other agents. In the first series 70 of 73 infected spleens were detected. There were no false positive reactions among the controls. Results obtained with the second series of pigs showed that spleens collected during advanced stages of the disease were more satisfactory specimens than those collected earlier during the high temperature phase of infection. Findings with the third series of older swine indicated that their spleens were less satisfactory as a source of virus than those from young pigs. Tissues from freshly killed animals provided better specimen material than those from animals that had died.

5912

Sirbu, Z.; Ieremia, D.; Bona, C. 1964. Immunofluorescent microscopy in the diagnosis of swine fever. *Brit. Vet. J.* 120:587-591.

The presence of virus was detected in lymph nodes as early as 60 hours after infection. Initially, the virus was intracytoplasmic, later it was observed in the nucleus. Results of tests were the same with animals from a natural outbreak and experimentally infected pigs. BA-46-108750.

5913

Stair, E.L.; Rhodes, M.B.; Aiken, J.M.; Underdahl, N.R.; Young, G.A. 1963. A hog cholera virus-fluorescent antibody system: Its potential use in study of embryonic infection. *Proc. Soc. Exp. Biol. Med.* 113:656-660.

A system of hog cholera virus and fluorescent antibody has been developed, using pathogen-free pigs as the source of globulins. Heterologous globulin was developed by hyperimmunizing pathogen-free genetically small pigs with three strains of virulent hog cholera virus. Success of the system appeared to be based on use of pigs with minimum non-specific globulins, complement for antigen-antibody union, and globulins that approach maximum levels of conjugation to fluorescein isothiocyanate. Use of this system in the study of viral infections of swine embryos and in rapid diagnosis of hog cholera is discussed.

5914

Tuomi, J.; Kangas, J. 1963. A fluorescent antibody technique for studies of mink virus enteritis. *Arch. Ges. Virusforsch.* 13:430-434.

A procedure is described for staining of FA mink virus enteritis antigens in the epithelial cells of the small intestines of experimentally infected mink. The specific viral antigens in these epithelial cells were confined to cytoplasmic bodies. The possible usefulness of the fluorescent antibody technique as a research and diagnostic tool in mink virus enteritis is discussed.

5915

Watson, B.B.; Eveland, W.C. 1965. The application of phage-fluorescent antiphage staining in the specific detection of Listeria monocytogenes in experimentally infected animal tissues. *Bacteriol. Proc.* M132:61.

Phage-fluorescent antiphage staining utilizes species-specific L. monocytogenes phages combined with the sensitivity of the immunofluorescent reaction in the detection of phage after attachment. This is an indirect technique in that bacteria are outlined by staining. Previous studies had shown this staining, with the use of smear preparations, to be highly specific and sensitive. These studies were extended to include its possible application in identification of L. monocytogenes in infected tissues. Infected and normal tissue sections were exposed for 3 hours to 100 million plaque-forming units per 0.1 ml of phage, washed with buffered saline, stained with conjugated phage-specific antiserum, and observed. Staining with conjugated listerial antiserum and two histochemical stains were also used to detect organisms in serial sections. A high correlation was found between phage-fluorescent antiphage and conjugated listerial antiserum, although fewer tissue-associated organisms were observed to fluoresce after staining by the former.

5916

Watson, B.B.; Eveland, W.C. 1965. The application of the phage-fluorescent antiphage staining system in the specific identification of Listeria monocytogenes: I. Species-specificity and immunofluorescent sensitivity of Listeria monocytogenes phage observed in smear preparations. *J. Infect. Dis.* 115:363-369.

A procedure has been described and results presented on the application of the phage-fluorescent antiphage staining system as an indirect technique in the identification of Listeria monocytogenes. Within the limits of tests performed on 63 strains of L. monocytogenes, staining reactions appeared to be both sensitive and specific. Both phage-sensitive and to a lesser degree phage-resistant strains showed fluorescence. All heterologous species of bacteria tested were negative. The staining pattern was distinct from that of ordinary methods of fluorescent staining by antibacterial serum in that organisms appeared irregular and often bizarre. A method of indirectly observing phage attachment by dark-field illumination is discussed.

5917

Wiktor, T.J.; Fernandes, M.V.; Koprowski, H. 1965. Detection of a lymphocytic choriomeningitis component in rabies virus preparations. *J. Bacteriol.* 90:1494-1495.

Tissue cultures chronically infected with rabies virus were examined by direct and indirect FA. Lymphocytic choriomeningitis virus was

found in 9 of 11 rabies-infected tissue cultures. Similar tissue cultures, either noninfected or infected with other viruses, did not yield lymphocytic choriomeningitis virus. This is thought not to be a casual relationship.

5918

Wilsnack, R.E.; Rowe, W.P. 1964. Immunofluorescent studies of the histopathogenesis of lymphocytic choriomeningitis virus infection. J. Exp. Med. 120:829-840.

LCM virus antigen was visualized in infected tissue cultures and mouse tissues by the direct immunofluorescent procedure. In all instances virus was localized in the cytoplasm. In intracerebrally infected mice, antigen was almost completely confined to meninges, choroid plexus, and ependyma; at no time were neurons involved. Mice infected by intraperitoneal inoculation of a viscerotropic strain demonstrated antigen chiefly in liver parenchyma, splenic reticulum, bronchi, and alveolar cells. Congenitally infected mice showed antigen in almost all cell types but generally in only a minority of cells; infection was often focal. Liver, kidney, pregnant uterus, and trophoblast were most extensively involved. No antigen was observable in ova of a pregnant mouse.

5919

Worley, J.F.; Schneider, I.R. 1963. Progressive distribution of southern bean mosaic virus antigen in bean leaves determined with a fluorescent antibody stain. Phytopathology 53:1255-1257.

Lyophilized, chloroform-fixed sections of plant material were used in conjunction with the fluorescent antibody stain technique to study the movement and distribution of southern bean mosaic virus antigen in bean plants. A specific FA stain indicated that, after the original infections are established in the epidermal cells of an inoculated primary leaf, contiguous cells become progressively infected. Staining of the opposite uninoculated leaf did not reveal such cell-to-cell progression, but instead the stain was first observed throughout the mesophyll.

B. BACTERIOPHAGE, INSECT VIRUS, PLANT VIRUS

5920

Bellett, A.J.D. 1965. The multiplication of Sericesthis iridescent virus in cell cultures from Antheraea eucalypti Scott: II. An in vitro assay for the virus. *Virology* 26:127-131.

The optimum temperature for production of the antigen of Sericesthis iridescent virus (SIV) by infected cell cultures was 20 C. Virus titers in infective units (IU) per ml were estimated from the proportion of cells that stained with fluorescent antibody 6.5 days after inoculation with dilutions of SIV. The proportion of uninfected cells was close to that predicted by the Poisson distribution at multiplicities below 0.5 IU per cell. A plot of virus titer against virus concentration was linear and passed through the origin. The error of the method was estimated. The ratio of particles to infectivity was about 80:1 for purified virus.

5921

Bellett, A.J.D. 1965. The multiplication of Sericesthis iridescent virus in cell cultures from Antheraea eucalypti Scott: III. Quantitative experiments. *Virology* 26:132-141.

SIV was not dissociated from infected Antheraea eucalypti cells by washing at times later than 10 hours after inoculation. Viral DNA was synthesized in cytoplasmic foci that also contained antigen. Each infective unit (IU) of virus formed a separate focus, but supernumerary foci were formed in some cells. Once replication was initiated in a multiply infected cell, all the foci began to operate at about the same time. Synthesis of viral DNA and antigen was detected in some cells 2 days after inoculation; most infected cells had begun viral replication by 6 days and continued to synthesize viral DNA for an additional 2 days. Inhibition of host cell nuclear DNA synthesis was not a prerequisite for viral DNA synthesis. Infective virus was eclipsed for about 4 days after inoculation and was then continuously produced and released from the cells until the 8th day. The yield was about 500 IU per infected cell, of which about 360 IU per cell were released. SIV was ether-resistant. It is proposed that iridescent viruses form a group separate from adenoviruses. FA was used to follow viral infection of the cells.

5922

Bellett, A.J.D.; Mercer, E.H. 1964. The multiplication of Sericesthis iridescent virus in cell cultures from Antheraea eucalypti Scott: I. Qualitative experiments. *Virology* 24:645-653.

Cultures of the Grace Antheraea eucalypti cells have been successfully infected with Sericesthis iridescent virus (SIV). Virus antigen and DNA, shown by fluorescent antibody and acridine orange staining, respectively, accumulated in discrete foci in the cytoplasm of infected cells. Cells containing such foci were first seen about 2 days after infection at 25 or 3 days at 21 C; the proportion of cells that contained foci increased to reach a maximum after 4 days of incubation at 25 or 6 days at 21 C. The size and intensity of fluorescence of the foci increased until the cytoplasm of many cells was filled with viral products. The cytoplasmic foci were shown by electron microscopy to contain aggregations of complete virus particles, developmental forms resembling those of Tipula iridescent virus, and diffuse material that stained with uranyl acetate. Single virus particles were also present elsewhere in the cytoplasm and in long processes extending from the cell surface. They appeared to separate from these by a budding process and acquired an envelope derived from the cell membrane. Seven consecutive passages of SIV in A. eucalypti cell cultures were successful at 21 C, but infectivity was lost after two passages at 25 C.

5923

Beumer-Jochmans, M.P. 1963. Characterization of pools of protein in cells of Shigella flexneri F6S infected with phage H-Sh. *Nature* 198:506-507.

Lesions in S. flexneri F6S following infection by phage H-Sh were studied by staining with acridine orange, coriphosphine, FA anti-bacterial serum, and FA anti-phage serum. The RNA and DNA patterns are described. Comparisons of the developmental progression of staining by the two FA reagents are made. Anti-bacterial staining is total and immediate. Anti-phage staining is spotty and progressive with time until burst of the bacteria.

5924

Hirai, T.; Hirai, A. 1964. Tobacco mosaic virus: Cytological evidence of the synthesis in the nucleus. *Science* 145:589-591.

Living tomato hair cells were directly injected with tobacco mosaic virus or tobacco mosaic virus RNA with a fine glass needle. Use of a fluorescent tobacco mosaic virus antibody showed the presence of the virus protein in the nucleus 6 to 18 hours after the injection. As time of infection progressed, staining of the cytoplasm became more prominent.

5925

Krywienczyk, J. 1963. Demonstration of nuclear polyhedrosis in Bombyx mori (Linnaeus) by fluorescent antibody technique. J. Insect Pathol. 5:309-317.

FA was adapted to demonstrate the presence of nuclear polyhedrosis in larvae of the silkworm Bombyx mori. Fluorescence was observed in the cytoplasm before crystallization of the polyhedra. Fluorescing polyhedra were found only in the nuclei.

5926

Nagaraj, A.N. 1965. Immunofluorescence studies on synthesis and distribution of tobacco mosaic virus antigen in tobacco. Virology 25:133-140.

Specific FA reagents were used to stain tobacco mosaic antigen (TMV) in plant tissue. Mesophyll cells and paraffin sections of leaves were FA-stained. Latent periods studied by FA or infectivity assay were similar. TMV antigen was first detected within the phloem and secondary xylem. In stem tips of infected plants, cells containing virus antigen were unevenly distributed. It was demonstrated that a strain of TMV hitherto believed to be a proteinless mutant produced TMV protein in infected cells.

5927

Nutter, R.L.; Woodruff, P. 1965. Quantitative specific antigen determination using fluorescent antibody. J. Clin. Pathol. 18:817-820.

A technique is described for the quantitation of a specific protein in the presence of other proteins. The application of this method to the assay of bacteriophage T-2 coat protein is presented and discussed.

5928

Sinha, R.C. 1965. Sequential infection and distribution of wound-tumor virus in the internal organs of a vector after ingestion of virus. Virology 26:673-686.

Fluorescent antibody techniques were used to detect wound-tumor virus (WTV) antigens in the hemolymph, brain, salivary glands, intestines, Malpighian tubules, ovaries, fat body, and mycetomes of the viruliferous leafhopper Agallia constricta Van Duzee. No antigens could be detected in the male reproductive organs. WTV antigens were localized in the cytoplasmic particulates of hemocytes and of fat body cells. Particles that stain specifically, by the fluorescent antiserum, in the hemolymph of viruliferous leafhoppers could be pelleted by low-speed centrifugation.

5929

Sinha, R.C.; Black, L.M. 1963. Wound-tumor virus antigens in the internal organs of an insect vector. *Virology* 21:183-187.

A simple method of dissecting out the various internal organs of Agallia constricta Van Duzee is described. Wound-tumor virus antigens were detected by FA in hemolymph smears of viruliferous leafhoppers of both sexes and in the smears of the fat body from female transmitters. The fat body of only one male transmitter of ten tested was positive. When organs of the same kind from 11 to 20 exposed or viruliferous leafhoppers were pooled and extracts tested for wound-tumor soluble antigens by the precipitin ring test, a positive reaction was given by the fat body, intestine, and salivary glands of both sexes and by the ovaries. Malpighian tubules and the testes failed to show a positive reaction. Although the tests suggested that the highest virus antigen concentration is in the fat body, it seems probable that the initial site of infection is the intestinal tract. By squeezing a small drop of hemolymph onto a slide and applying the fluorescent smear test it is possible to determine, without damaging any of the organs, whether an insect is viruliferous.

5930

Sinha, R.C.; Reddy, D.V.R. 1964. Improved fluorescent smear technique and its application in detecting virus antigens in an insect vector. *Virology* 24:626-634.

Antiserum to purified wound tumor viruses (WTV) was prepared in rabbits. Ammonium sulfate globulin precipitation was found more effective at pH 7.4 than without adjusting the pH. The dialysis conjugation method at pH 9.8 was superior to conjugation by direct addition of dye to globulin solution. In experiments correlating results of smear tests of vectors and their transmission of virus to plants, 107 smears of exposed leafhoppers were stained with the D conjugates. Of these, 76 were positive and 24 negative both by smear test and by transmission. Seven leafhoppers were positive by smear test but did not transmit WTV in the transmission tests. On the other hand, there were no leafhoppers that were positive by transmission but negative by smear test. WTV could be detected earlier by smear test than by transmission. A method of scoring smears of leafhoppers in ultraviolet light without using a microscope is described.

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